

CELL CRYOPRESERVATION AND DELIVERY VEHICLE AND METHODS OF USING THE SAME

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority of U.S. Provisional Application No. 63/607,060, filed December 6, 2023, and U.S. Provisional Application No. 63/554,094, filed February 15, 2024, the entire contents of which are each incorporated herein by reference.

FIELD OF INVENTION

[0002] This disclosure relates generally to solutions for cryopreservation and delivery of cells to a subject.

BACKGROUND

[0003] Cell therapy has emerged as a promising approach for treating a wide range of medical conditions, including but not limited to, degenerative disorders (e.g., Parkinson's disease), heart failure, and autoimmune diseases. To facilitate the clinical application of cell therapies, it is often necessary to cryopreserve cells for long-term storage, transport, and subsequent administration to patients. Cryopreservation involves freezing cells at ultra-low temperatures to preserve their viability and functionality until needed.

[0004] Traditional cryopreservation methods typically involve the use of a cryoprotective solution to protect cells from damage during freezing and thawing processes. However, one drawback of existing cryopreservation and delivery methods is the need to wash cryopreserved cells thoroughly before administration to remove cryoprotective agents. This washing step can be time-consuming, labor-intensive, and may lead to cell loss or reduced cell viability.

SUMMARY

[0005] This disclosure relates to methods and compositions for cryopreserving and administering cells without the need for any post-thaw wash steps. This disclosure provides a direct "thaw-to-inject" approach for delivering therapeutic cell populations. The formulations/solutions and methods disclosed herein allow cryopreserved cells to be

administered to patients directly by a workflow that bypasses the time-consuming washing processes which are typically required in traditional cell therapy. As a result, it enhances the efficiency, safety, and overall clinical success rates of cell therapies.

[0006] In one aspect, this disclosure provides a cell cryopreservation and delivery solution, the solution comprising: about 25 mM to about 280 mM of one or more energy source components; about 10 mM to about 200 mM of one or more salts; and about 1% wt to about 30% wt of one or more cryoprotectants. In some embodiments, the cell cryopreservation and delivery solution is formulated such that the solution can be used to administer a population of cells to a subject in need thereof without the need for any post-thaw wash steps.

[0007] In some embodiments, the cell cryopreservation and delivery solution further comprises about 5 mM to about 40 mM of one or more pH buffers. In some embodiments, the one or more pH buffers comprise 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 3-(N-morpholino)propanesulfonic acid (MOPS), 2-(N-morpholino) ethanesulfonic acid (MES), bicarbonate, or phosphates.

[0008] In some embodiments, further comprising about 0.01% wt to about 25% wt of one or more stabilizing agents. In some embodiments, the one or more stabilizing agents comprise one or more nonpermeating-type cryoprotectants. In some embodiments, the one or more stabilizing agents comprise dextran, poloxamer, polyvinylpyrrolidone, one or more antioxidants, isoleucine, or albumin.

[0009] In some embodiments, the cell cryopreservation and delivery solution comprise one or more antioxidants. In some embodiments, the one or more antioxidants comprise glutathione, isoleucine or proline. In some embodiments, the one or more antioxidants comprise glutathione.

[0010] In some embodiments, the one or more cryoprotectants comprise propylene glycol, glycerol, or dimethyl sulfoxide (DMSO). In some embodiments, the one or more cryoprotectants comprise propylene glycol.

[0011] In some embodiments, the one or more energy source components comprise dextrose, glucose, sucrose, mannitol or trehalose.

[0012] In some embodiments, the one or more salts comprise one or more of calcium chloride, magnesium chloride, potassium chloride, sodium chloride, or sodium phosphate.

[0013] In some embodiments, the cell cryopreservation and delivery solution comprises: about 120 mM to about 190 mM glucose; about 4 mM to about 40 mM HEPES; about 0.1 mM to about 2.5 mM calcium chloride; about 0.1 mM to about 1.5 mM magnesium chloride; about 4.0 mM to about 6.5 mM potassium chloride; about 20 mM to about 150 mM sodium chloride; about 0.1% wt to about 20% wt dextran; and about 5% wt to about 25% wt of the one or more cryoprotectants.

[0014] In some embodiments, the cell cryopreservation and delivery solution comprises about 145 mM to about 165 mM glucose; about 10 mM to about 15 mM HEPES; about 0.1 mM to about 2.5 mM calcium chloride; about 0.5 mM to about 1.0 mM magnesium chloride; about 4.0 mM to about 6.0 mM potassium chloride; about 60 mM to about 80 mM sodium chloride; about 0.1% wt to about 10% wt Dextran; and about 10% wt to about 15% wt of one or more cryoprotectants. In some embodiments, the one or more cryoprotectants comprise dimethyl sulfoxide (DMSO). In some embodiments, the one or more cryoprotectants comprise propylene glycol.

[0015] In some embodiments, the cell cryopreservation and delivery solution does not include any components of animal origin. In some embodiments, the solution does not include one or more of zinc sulfate, and ferric nitrate.

[0016] In some embodiments, the cell cryopreservation and delivery solution comprises a pH between about 7.0 and about 8.0. In some embodiments, the pH indicator is less than 7.8.

[0017] In some embodiments, the cell cryopreservation and delivery solution does not include sodium pyruvate.

[0018] In some embodiments, the cell cryopreservation and delivery solution enables cryopreservation of a plurality of cells at a therapeutically effective dose. In some embodiments, the cell cryopreservation and delivery solution the cell cryopreservation and delivery solution enables stable storage of a plurality of cells for up to at least about 6 hours at 4 °C.

[0019] In another aspect, this disclosure provides a cell suspension for cryopreservation and administration to a subject, the suspension comprising: a plurality of cells; about 25 mM to about 280 mM of one or more energy source components; about 10 mM to about 200 mM of one or more salts; and about 1% wt to about 30% wt of one or more cryoprotectants.

[0020] In some embodiments, the plurality of cells comprise human pluripotent stem cells, embryonic stem cells, cardiac cells, microglial cells, T-cells, or neural cells. In some embodiments, the cells comprise neural cells and wherein the neural cells comprise dopaminergic neuronal precursor cells.

[0021] In some embodiments, the plurality of cells comprises a population of about 10^4 cells/mL to about 10^{10} cells/mL. In some embodiments, the plurality of cells comprises a range of about 10^5 cells/mL to about 1.5×10^8 cells/mL. In some embodiments, the suspension does not require contacting the plurality of cells with a cell wash solution prior to administration to the subject. In some embodiments, the cell wash solution comprises a phosphate buffer saline (PBS), Bio-Plex Pro™ Cell Signaling Wash Buffer, or Cultrex™ 3-D Cell Wash Buffer.

[0022] In some embodiments, the suspension further comprises about 5 mM to about 40 mM of one or more pH buffers. In some embodiments, the suspension further comprises about 0.01% wt to about 25% wt of one or more stabilizing agents.

[0023] In some embodiments, the one or more cryoprotectants of the suspension comprises propylene glycol, glycerol, or dimethyl sulfoxide (DMSO). In some embodiments, the one or more cryoprotectants comprise propylene glycol.

[0024] In some embodiments, the one or more energy source components of the suspension comprise dextrose, glucose, sucrose, mannitol or trehalose. In some embodiments, the one or more pH buffers comprise 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 3-(N-morpholino)propanesulfonic acid (MOPS), 2-(N-morpholino) ethanesulfonic acid (MES), bicarbonate, or phosphates.

[0025] In some embodiments, the one or more salts of the suspension comprise one or more of calcium chloride, magnesium chloride, potassium chloride, sodium chloride, or sodium phosphate. In some embodiments, the one or more stabilizing agents of the

suspension comprise dextran, poloxamer, polyvinylpyrrolidone, one or more antioxidants, or albumin.

[0026] In some embodiments, the suspension comprises: about 120 mM to about 190 mM glucose; about 4 mM to about 40 mM HEPES; about 0.1 mM to about 2.5 mM calcium chloride; about 0.1 mM to about 1.5 mM magnesium chloride; about 4.0 mM to about 6.5 mM potassium chloride; about 20 mM to about 150 mM sodium chloride; about 0.1% wt to about 20% wt dextran; and about 5% wt to about 25% wt of the one or more cryoprotectants.

[0027] In some embodiments, the suspension comprises: about 145 mM to about 165 mM glucose; about 10 mM to about 15 mM HEPES; about 0.1 mM to about 2.5 mM calcium chloride; about 0.5 mM to about 1.0 mM magnesium chloride; about 4.0 mM to about 6.0 mM potassium chloride; about 60 mM to about 80 mM sodium chloride; about 0.1% wt to about 10% wt dextran; and about 10% wt to about 15% wt of one or more cryoprotectants.

[0028] In some embodiments, the one or more cryoprotectants of the suspension comprise dimethyl sulfoxide (DMSO). In some embodiments, the one or more cryoprotectants of the suspension comprise propylene glycol.

[0029] In some embodiments, the suspension does not include any components of animal origin. In some embodiments, the suspension does not include one or more of zinc sulfate, and ferric nitrate.

[0030] In some embodiments, the suspension comprises a pH between about 7.0 and about 8.0. In some embodiments, the pH is below 7.8. In some embodiments, the suspension does not include sodium pyruvate.

[0031] In some embodiments, the suspension comprises one or more antioxidants. In some embodiments, the one or more antioxidants comprise glutathione, isoleucine or proline. In some embodiments, the one or more antioxidants comprise glutathione.

[0032] In some embodiments, the cell suspension enables cryopreservation of the plurality of cells at a therapeutically effective dose. In some embodiments, the cell

suspension enables stable storage of the plurality of cells for up to at least about 6 hours at 4 °C.

[0033] In another aspect, this disclosure provides a method of preparing a cell suspension for cryopreservation and administration to a subject, the method comprising: adding a cell cryopreservation and delivery solution described herein to a plurality of cells in a container; and mixing the plurality of cells and the cell cryopreservation and delivery solution to form a cell suspension. In some embodiments, the plurality of cells comprises a population of about 10^4 cells/mL to about 10^{10} cells/mL. In some embodiments, the plurality of cells comprises a range of about 10^5 cells/mL to about 1.5×10^8 cells/mL.

[0034] In some embodiments, the volume of the cell suspension is about 0.1 mL to about 20 mL. In some embodiments, the volume of the cell suspension is about 20 mL. In some embodiments, the plurality of cells has a variability in dose sampling of less than about 25% in the container.

[0035] In some embodiments, the cells are differentiated midbrain dopaminergic neuronal cells, or a precursor thereof.

[0036] In some embodiments, the method further comprises cryopreserving the cell suspension. In some embodiments, the method further comprises thawing the cryopreserved cell suspension, and administering the thawed cell suspension to a subject. In some embodiments, the method does not comprise washing the cells with a cell wash solution after thawing the cells. In some embodiments, the method does not include using a cell wash solution, for example, any one of phosphate buffer saline (PBS), Bio-Plex Pro™ Cell Signaling Wash Buffer, or Cultrex™ 3-D Cell Wash Buffer.

[0037] In another aspect, this disclosure provides a method of treating a subject, the method comprising: thawing a cell suspension described herein; and administering a dose of the cell suspension to the subject, wherein the method does not comprise a wash step.

[0038] In some embodiments, the cells are differentiated midbrain dopaminergic neuronal cells. In some embodiments, the plurality of cells comprises a population of about 10^4 cells/mL to 10^{10} cells/mL. In some embodiments, wherein the population comprises about 10^5 cells/mL to about 1.5×10^8 cells/mL.

[0039] In some embodiments, the method further comprises the step of administering one or more additional doses of the cell suspension. In some embodiments, the administering comprises loading a dose delivery device with the dose of the cell suspension, and injecting the dose to the subject. In some embodiments, the injecting occurs directly into a portion of the brain of the subject. In some embodiments, the portion comprises a putamen. In some embodiments, the dose delivery device is a syringe. In some embodiments, wherein the subject is a human. In some embodiments, the subject has Parkinson's disease. In some embodiments, the Parkinson's disease is advanced Parkinson's disease.

[0040] In another aspect, this disclosure provides a method of treating a subject, the method comprising: thawing a cell suspension comprising about 1% wt to about 30% wt of one or more cryoprotectants; and administering a dose of the cell suspension to the subject.

[0041] In some embodiments, the method does not comprise a cell wash step.

[0042] In some embodiments, the method does not comprise diluting the cell suspension prior to the administering.

[0043] In some embodiments, the cell suspension comprises about 25 mM to about 280 mM of one or more energy source components; about 10 mM to about 200 mM of one or more salts; and about 1% wt to about 30% wt of the one or more cryoprotectants.

[0044] In some embodiments, the cell suspension further comprises about 5 mM to about 40 mM of one or more pH buffers. In some embodiments, the cell suspension further comprises one or more pH buffers, wherein the one or more pH buffers comprise 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 3-(N-morpholino)propanesulfonic acid (MOPS), 2-(N-morpholino)ethanesulfonic acid (MES), bicarbonate, or phosphates.

[0045] In some embodiments, the cell suspension further comprises about 0.01% wt to about 25% wt of one or more stabilizing agents.

[0046] In some embodiments, the one or more stabilizing agents comprise dextran, poloxamer, polyvinylpyrrolidone, one or more antioxidants, or albumin.

[0047] In some embodiments, the one or more cryoprotectants comprise propylene glycol, glycerol, or dimethyl sulfoxide (DMSO). In some embodiments, the one or more cryoprotectants comprise propylene glycol.

[0048] In some embodiments, the one or more energy source components comprise dextrose, glucose, sucrose, mannitol or trehalose. In some embodiments, the one or more salts comprise one or more of calcium chloride, magnesium chloride, potassium chloride, sodium chloride, or sodium phosphate.

[0049] In some embodiments, the cell suspension comprises: about 120 mM to about 190 mM glucose; about 4 mM to about 40 mM HEPES; about 0.1 mM to about 2.5 mM calcium chloride; about 0.1 mM to about 1.5 mM magnesium chloride; about 4.0 mM to about 6.5 mM potassium chloride; about 20 mM to about 150 mM sodium chloride; about 0.1% wt to about 20% wt dextran; and about 5% wt to about 25% wt of the one or more cryoprotectants.

[0050] In some embodiments, the cell suspension comprises: about 145 mM to about 165 mM glucose; about 10 mM to about 15 mM HEPES; about 0.1 mM to about 2.5 mM calcium chloride; about 0.5 mM to about 1.0 mM magnesium chloride; about 4.0 mM to about 6.0 mM potassium chloride; about 60 mM to about 80 mM sodium chloride; about 0.1% wt to about 10% wt dextran; and about 10% wt to about 15% wt of one or more cryoprotectants.

[0051] In some embodiments, the cell suspension comprises a cryopreservation solution comprising one or more cryoprotectants selected from the group consisting of dimethyl sulfoxide (DMSO), DMSO-free cryoprotectants, and combinations thereof.

[0052] In some embodiments, the cryopreservation solution comprises: about 5-15% DMSO; or about 5% to about 15% propylene glycol and about 1% to about 5% dextran 40.

[0053] In some embodiments, the cell suspension comprises one of STEM-CELLBANKER™ EX, STEM-CELLBANKER™, STEM-CELLBANKER™ DMSO Free, or XT-THRIVE™.

[0054] In another aspect, this disclosure provides a method of preparing a cell cryopreservation and delivery solution, the method comprising: preparing a first solution comprising about 0.2% wt to about 20% wt dextran and about 20% wt to about 30% wt of one or more cryoprotectants; preparing a second solution comprising: about 145 mM to about 165 mM glucose, about 10 mM to about 15 mM HEPES, about 0.1 mM to about 2.5 mM

calcium chloride, about 0.5 mM to about 1.0 mM magnesium chloride, about 4.0 mM to about 6.0 mM potassium chloride, and about 60 mM to about 80 mM sodium chloride; and combining substantially equal volumes of the first solution and the second solution to form the cell cryopreservation and delivery solution.

[0055] In another aspect, this disclosure provides a method of preparing a final cell suspension for cryopreservation and delivery to a subject, the method comprising: suspending a plurality of cells in an isotonic solution to form a cell suspension; preparing a concentrated cryoprotectant solution comprising about 20% wt to about 30% wt of one or more cryoprotectants; and gradually adding the concentrated cryoprotectant solution to the cell suspension to form the final cell suspension. In some embodiments, the isotonic solution comprises about 145 mM to about 165 mM glucose, about 10 mM to about 15 mM HEPES, about 0.1 mM to about 2.5 mM calcium chloride, about 0.5 mM to about 1.0 mM magnesium chloride, about 4.0 mM to about 6.0 mM potassium chloride, and about 60 mM to about 80 mM sodium chloride. In some embodiments, the concentrated cryoprotectant solution further comprises about 0.2% wt to about 20% wt dextran.

[0056] In another aspect, this disclosure provides a method of treating a subject, the method comprising: thawing a cell suspension comprising about 1% wt to about 30% wt of one or more cryoprotectants, and administering a dose of the cell suspension to the subject.

[0057] In some embodiments, the method does not comprise a cell wash step. For example, in some embodiments, the method does not comprise contacting the cell suspension with a cell wash solution (*i.e.*, any solution that is used for washing the cells post thaw, for example, to remove the cryopreservation solution from cell suspension) after thawing the cell suspension and before administering a dose of the cell suspension to the subject. In some embodiments, the method does not comprise diluting the cell suspension prior to the administering. In some embodiments, the cell suspension comprises a cryopreservation and delivery solution as described herein.

[0058] In one aspect, provided herein are cell cryopreservation and delivery solutions. In some embodiments, the cell cryopreservation and delivery solution comprises about 25 mM to about 280 mM of one or more energy source components; about 10 mM to about 200 mM of one or more salts; and about 1% wt to about 30% wt of a one or more cryoprotectants.

[0059] In some embodiments, the one or more cryoprotectants comprise propylene glycol or dimethyl sulfoxide (DMSO). In some embodiments, the one or more cryoprotectants comprise propylene glycol. In some embodiments, the one or more energy source components comprise dextrose or glucose. In some embodiments, the one or more salts comprise one or more of calcium chloride, magnesium chloride, potassium chloride, sodium chloride, or sodium phosphate.

[0060] In some embodiments, the cell cryopreservation and delivery solution further comprises about 5 mM to about 40 mM of one or more pH buffers. In some embodiments, the one or more pH buffers comprise 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 3-(N-morpholino)propanesulfonic acid (MOPS), 2-(N-morpholino) ethanesulfonic acid (MES), bicarbonate, or phosphates.

[0061] In some embodiments, the cell cryopreservation and delivery solution further comprises about 0.01% wt to about 25% wt of one or more stabilizing agents. In some embodiments, the one or more stabilizing agents comprise dextran, poloxamer, polyvinylpyrrolidone, an antioxidant (e.g., reduced glutathione) or albumin.

[0062] In some embodiments, the cell cryopreservation and delivery solution comprises: about 20 mM to about 30 mM dextrose; about 10 mM to about 50 mM HEPES; about 0.1 mM to about 2.5 mM calcium chloride; about 0.1 mM to about 1.5 mM magnesium chloride; about 4.5 mM to about 6.5 mM potassium chloride; about 40 mM to about 150 mM sodium chloride; about 0.1 mM to about 1.5 mM sodium phosphate monobasic; about 5% wt to about 20% wt Dextran; about 0.01% wt to about 20% wt Poloxamer; about 0.05% wt to about 0.5% recombinant human serum albumin; and about 5% wt to about 20% wt of the one or more cryoprotectants. In some embodiments, the cell cryopreservation and delivery solution comprises sodium phosphate dibasic. For example, in some embodiments, the cell cryopreservation and delivery solution comprises about 0.1 to about 15 mM sodium dibasic.

[0063] In some embodiments, the cell cryopreservation and delivery solution comprises: about 22.1 mM dextrose; about 9.7 mM HEPES; about 1.6 mM calcium chloride; about 0.7 mM magnesium chloride; about 4.8 mM potassium chloride; about 75 mM sodium chloride; about 0.7 mM sodium phosphate monobasic; about 15.9% wt dextran; about 0.07% wt poloxamer; and about 10% wt of the one or more cryoprotectants. In some embodiments,

the one or more cryoprotectants comprise dimethyl sulfoxide (DMSO). In some embodiments, the one or more cryoprotectants comprise propylene glycol.

[0064] In some embodiments, the cell cryopreservation and delivery solution comprises a pH indicator. In some embodiments, the pH indicator is Phenol Red.

[0065] In some embodiments, the cell cryopreservation and delivery solution does not include any components of animal origin. In some embodiments, the cell cryopreservation and delivery solution does not include one or more of zinc sulfate, and ferric nitrate. In some embodiments, the cell cryopreservation and delivery solution does not include sodium pyruvate.

[0066] In some embodiments, the cell cryopreservation and delivery solution further comprises one or more excipients selected from the group consisting of polyethylene glycol, carboxymethyl cellulose, hyaluronic acid, starches, acrylates, methacrylates, polyvinyl alcohols, polyethylene oxides, polypropylene oxides, polyacrylates, polyvinylpyrrolidone, polymethacrylate, polylactic-co-glycolic acids, polyacrylamides, polylactides, chitosans, gums, guar gums, xantham gums, carrageenans, hydroxypropyl cellulose, hydroxypropyl methyl cellulose, hydroxyethyl cellulose, cyclodextrin derivatives, beta-cyclodextrin derivatives, alginates, calcium alginates, dextran, pentaisomaltose, hydroxyethyl starch, trehalose, sorbitol, mannitol, reduced glutathione, isoleucine, proline, ascorbic acid, N-acetyl-L-cysteine, carnitine, betaine, glycolipids, PEG lipids, lipoprotein, polysorbate 20, polysorbate 80, polyoxyl 15 hydroxy stearate, ectoine, and stearates.

[0067] In some embodiments, the cell cryopreservation and delivery solution comprises an additional cryoprotectant. In some embodiments, the additional cryoprotectant is a permeating-type cryoprotectant. In some embodiments, the permeating-type cryoprotectant is selected from the group consisting of dimethyl sulfoxide (DMSO), glycerol, and propylene glycol.

[0068] In some embodiments, the cell cryopreservation and delivery solution enables cryopreservation of a plurality of cells at a therapeutically effective dose. In some embodiments, the cell cryopreservation and delivery solution enables stable storage of a plurality of cells for up to at least about 6 hours at 4 °C.

[0069] In another aspect, provided herein are cell suspensions for cryopreservation and administration to a subject. In some embodiments, the suspension comprises: a plurality of cells; about 0.5 mM to about 280 mM of one or more energy source components; about 0.1 mM to about 200 mM of one or more salts; and about 5% wt to about 20% wt of one or more cryoprotectants.

[0070] In some embodiments, the cells are differentiated midbrain dopaminergic neuronal cells, or a precursor thereof. In some embodiments, the differentiated midbrain dopaminergic neuronal cells are differentiated midbrain dopaminergic neuron progenitor cells.

[0071] In some embodiments, the plurality of cells comprises a range of about 10^0 cells to about 10^{10} cells. In some embodiments, the plurality of cells comprises a range of about 10^5 cells to about 10^8 cells.

[0072] In some embodiments, the suspension does not require contacting the plurality of cells with a cell wash buffer (*i.e.*, any solution that is used for washing the cells post thaw, for example, to remove the cryopreservation solution from cell suspension) prior to administration to the subject. In some embodiments, the cell wash buffer is selected from the group consisting of a phosphate buffer saline (PBS), Bio-Plex Pro™ Cell Signaling Wash Buffer, and Cultrex™ 3-D Cell Wash Buffer.

[0073] In some embodiments, the one or more cryoprotectants comprise propylene glycol or dimethyl sulfoxide (DMSO). In some embodiments, the one or more cryoprotectants comprise propylene glycol. In some embodiments, the one or more energy source components comprise dextrose or glucose. In some embodiments, the one or more salts comprise one or more of calcium chloride, magnesium chloride, potassium chloride, sodium chloride, or sodium phosphate.

[0074] In some embodiments, the suspension further comprises about 5 mM to about 50 mM of one or more pH buffers. In some embodiments, the one or more pH buffers comprise 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 3-(N-morpholino)propanesulfonic acid (MOPS), 2-(N-morpholino) ethanesulfonic acid (MES), bicarbonate, or phosphates.

[0075] In some embodiments, the suspension further comprises about 0.01% wt to about 25% wt of one or more stabilizing agents. In some embodiments, the one or more stabilizing agents comprise dextran, poloxamer, polyvinylpyrrolidone, an antioxidant (e.g., reduced glutathione) or albumin.

[0076] In some embodiments, the suspension comprises: about 20 mM to about 50 mM dextrose; about 10 mM to about 50 mM HEPES; about 0.1 mM to about 2.5 mM calcium chloride; about 0.1 mM to about 1.5 mM magnesium chloride; about 4.5 mM to about 6.5 mM potassium chloride; about 40 mM to about 150 mM sodium chloride; about 0.1 mM to about 1.5 mM sodium phosphate monobasic; about 5% wt to about 20% wt Dextran; about 0.01% wt to about 20% wt Poloxamer; about 0.05% wt to about 0.5% recombinant human serum albumin; and about 5% wt to about 20% wt of the one or more cryoprotectants. In some embodiments, the suspension further comprises sodium phosphate dibasic. For example, in some embodiments, the suspension comprises about 0.1 to about 15 mM sodium dibasic.

[0077] In some embodiments, the suspension comprises: about 22.1 mM dextrose; about 9.7 mM HEPES; about 1.6 mM calcium chloride; about 0.7 mM magnesium chloride; about 4.8 mM potassium chloride; about 75 mM sodium chloride; about 0.7 mM sodium phosphate monobasic; about 15.9% wt Dextran; about 0.07% wt Poloxamer; and about 10% wt of the one or more cryoprotectants. In some embodiments, the one or more cryoprotectants comprise dimethyl sulfoxide (DMSO). In some embodiments, the one or more cryoprotectants comprise propylene glycol.

[0078] In some embodiments, the suspension comprises a pH indicator. In some embodiments, the pH indicator is Phenol Red.

[0079] In some embodiments, the suspension does not include any components of animal origin. In some embodiments, the suspension does not include one or more of zinc sulfate, and ferric nitrate. In some embodiments, the suspension does not include sodium pyruvate.

[0080] In some embodiments, the suspension further comprises one or more excipients selected from the group consisting of polyethylene glycol, carboxymethyl cellulose, hyaluronic acid, starches, acrylates, methacrylates, polyvinyl alcohols, polyethylene oxides, polypropylene oxides, polyacrylates, polyvinylpyrrolidone,

polymethacrylate, poly lactic-co-glycolic acids, polyacrylamides, polylactides, chitosans, gums, guar gums, xantham gums, carrageenans, hydroxypropyl cellulose, hydroxypropyl methyl cellulose, hydroxyethyl cellulose, cyclodextrin derivatives, beta-cyclodextrin derivatives, alginates, calcium alginates, dextran, pentaisomaltose, hydroxyethyl starch, trehalose, sorbitol, mannitol, reduced glutathione, isoleucine, proline, ascorbic acid, N-acetyl-L-cysteine, carnitine, betaine, glycolipids, PEG lipids, lipoprotein, polysorbate 20, polysorbate 80, polyoxyl 15 hydroxy stearate, ectoine, and stearates.

[0081] In some embodiments, the suspension further comprises an additional cryoprotectant. In some embodiments, the additional cryoprotectant is a permeating-type cryoprotectant. In some embodiments, the permeating-type cryoprotectant is selected from the group consisting of dimethyl sulfoxide (DMSO), glycerol, and ethylene glycol.

[0082] In some embodiments, the cell suspension enables cryopreservation of the plurality of cells at a therapeutically effective dose. In some embodiments, the cell suspension enables stable storage of the plurality of cells for up to at least about 6 hours at 4 °C.

[0083] In yet another aspect, provided herein are methods of preparing a cell suspension for cryopreservation and administration to a subject. In some embodiments, the method for preparing a cell suspension for cryopreservation and administration to a subject comprises adding a cell cryopreservation and delivery solution of provided herein to a plurality of cells in a container; and mixing the plurality of cells and the cell cryopreservation and delivery solution to form a cell suspension.

[0084] In some embodiments, the plurality of cells comprises a range of about 10^0 cells to about 10^{10} cells. In some embodiments, the plurality of cells comprises a range of about 10^5 cells to about 10^8 cells.

[0085] In some embodiments, the volume of the cell suspension is about 0.05 mL to about 20 mL.

[0086] In some embodiments, the plurality of cells has a variability in dose sampling of less than about 25% in the container.

[0087] In some embodiments, the cells are differentiated midbrain dopaminergic neuronal cells, or a precursor thereof.

[0088] In some embodiments, the method for preparing a cell suspension for cryopreservation and administration to a subject further comprises cryopreserving the cell suspension.

[0089] In some embodiments, the method for preparing a cell suspension for cryopreservation and administration to a subject further comprises thawing the cryopreserved cell suspension, and administering the thawed cell suspension to a subject. In some embodiments, the method does not comprise washing the cells with a cell wash buffer (*i.e.*, any solution that is used for washing the cells post thaw, for example, to remove the cryopreservation solution from cell suspension) after thawing the cells. In some embodiments, the cell wash buffer is selected from the group consisting of a phosphate buffer saline (PBS), Bio-Plex Pro™ Cell Signaling Wash Buffer, and Cultrex™ 3-D Cell Wash Buffer.

[0090] In yet another aspect, provided herein are methods for treating a subject. In some embodiments, the method for treating a subject comprises thawing a cell suspension provided herein; and administering a dose of the cell suspension to the subject, wherein the method does not comprise a wash step.

[0091] In some embodiments, the cells are differentiated midbrain dopaminergic neuronal cells. In some embodiments, the plurality of cells comprises a range of about 10^0 cells to 10^{10} cells. In some embodiments, the plurality of cells comprises a range of about 10^5 cells to about 10^8 cells.

[0092] In some embodiments, the method for treating a subject further comprises the step of administering one or more additional doses of the cell suspension.

[0093] In some embodiments, the method for treating a subject further comprises a concurrent step or a subsequent step of administering a treatment, the treatment selected from the group consisting of a chemotherapeutic agent, a radiation treatment, a bone marrow treatment, a hormone treatment, and a surgical treatment.

[0094] In some embodiments, the cell wash buffer is selected from, but not limited to, the group consisting of a phosphate buffer saline (PBS), Bio-Plex Pro™ Cell Signaling Wash Buffer, and Cultrex™ 3-D Cell Wash Buffer.

[0095] In some embodiments, the administering comprises loading a dose delivery device with the dose of the cell suspension, and injecting the dose to the subject. In some embodiments, the injecting occurs directly into a portion of the brain of the subject. In some embodiments, the portion comprises a putamen. In some embodiments, the injecting step occurs into an intravenous line, wherein the intravenous line is in a blood vessel of the subject. In some embodiments, the dose delivery device is a syringe.

[0096] In some embodiments, the subject is a human. In some embodiments, the subject has Parkinson's disease. In some embodiments, the Parkinson's disease is advanced Parkinson's disease.

BRIEF DESCRIPTION OF DRAWINGS

[0097] **FIGS. 1A-1C** show exemplary experimental results assessing cryopreservation and delivery solutions. **FIG. 1A** shows a comparison of live cell concentrations (cells/mL) for test solutions identified as F1-F26, cell delivery solution with propylene glycol (CDS + Propylene Glycol), STEM-CELLBANKER, and STEM-CELLBANKER DMSO-Free, as indicated along the x-axis. **FIG. 1B** is a bar graph showing the cell viability percentage (%) for the test solutions indicated on the x-axis. **FIG. 1C** is a bar graph showing the replating efficiency of cells following cryopreservation with the different test solutions identified on the x-axis.

[0098] **FIGS. 2A-2C** show exemplary experimental results assessing the effectiveness of cryopreservation and delivery solutions for storing and using neural cells. **FIG. 2A** and **FIG. 2B** shows a comparison of live cell concentration and cell viability percentage (%), respectively, of neural cells in of STEM-CELLBANKER (SCB), SCB DMSO free, cell delivery solution (CDS) + 10% DMSO, and CDS + 10% propylene glycol. **FIG. 2C** shows the replating efficiency of neural cells following cryopreservation with STEM-CELLBANKER (SCB), SCB DMSO free, cell delivery solution (CDS) + 10% DMSO, and CDS + 10% propylene glycol. As used in the figure: CSI, cell suspension for injection (control); SCB, STEM-CELLBANKER; SCB+DMSO free; STEM-CELLBANKER

dimethyl sulfoxide-free; CDS+DMSO, cell delivery solution with 10% dimethyl sulfoxide; and CDS+PG; cell delivery solution with 10% propylene glycol.

[0099] FIGS. 3A-3B show exemplary experimental results comparing different polymer types for cell cryopreservation and delivery solutions. FIG. 3A shows cell viability percentages (%) and FIG. 3B shows replating efficiency percentages (%) for different polymers of various molecular weights (polymers indicated along x-axis). Data points represent individual measurements with bars indicating mean values.

[0100] FIGS. 4A-4B show exemplary experimental results evaluating the impact of Dextran 40 concentration on cell preservation outcomes. FIG. 4A shows cell viability percentages (%) and FIG. 4B shows replating efficiency percentages (%) for varying concentrations of Dextran 40 (0%, 1%, 5%, 10%, and 15%). Data points represent individual measurements with bars indicating mean values.

[0101] FIGS. 5A-5D show exemplary experimental results evaluating polyvinylpyrrolidone in cell cryopreservation and delivery solutions. FIG. 5A shows exemplary data of cell viability percentages (%) comparing 1% versus 5% PVP K12 concentrations. FIG. 5B shows exemplary data of replating efficiency percentages (%) comparing 1% versus 5% PVP K12 concentrations. FIG. 5C shows exemplary data of cell viability percentage (%) for cells preserved with 5% PVP K30. FIG. 5D shows replating efficiency percentage (%) for cells preserved with 5% PVP K30. All data points represent individual measurements with bars indicating mean values.

[0102] FIGS. 6A-6B show exemplary experimental results comparing Dextran 40 versus Dextran 70 in cell cryopreservation and delivery solutions. FIG. 6A shows cell viability percentages (%) measured pre-cryopreservation, immediately after thawing (0 Hour), and after 6 hours post-thaw for formulations containing either 5% Dextran 40 or 5% Dextran 70 in combination with 10% propylene glycol. FIG. 6B shows replating efficiency percentages (%) at 0 and 6 hours post-thaw for the same formulations. Data are presented as mean values with error bars indicating standard deviation.

[0103] FIGS. 7A-7B show experimental results evaluating the effect of glucose concentration (3% versus 0.5%) in two different cell cryopreservation and delivery solution formulations. FIG. 7A shows post-thaw cell viability percentages (%) and FIG. 7B shows replating efficiency percentages (%) for each glucose concentration in Formulation A (10%

propylene glycol, 1% Dextran 40) and Formulation B (12.5% propylene glycol, 5% Dextran 40). Data points represent individual measurements with bars indicating mean values. Statistical significance is indicated by asterisk (*).

[00104] **FIGS. 8A-8B** show exemplary experimental results evaluating the effect of glucose concentration in cell cryopreservation and delivery solutions. **FIG. 8A** shows post-thaw cell viability percentages (%) and **FIG. 8B** shows replating efficiency percentages (%) comparing 3% versus 0.5% glucose in four exemplary cell cryopreservation and delivery solutions: Formulation #47 (10% propylene glycol, 1% Dextran 40, 3% glucose), Formulation #49 (10% propylene glycol, 1% Dextran 40, 0.5% glucose), Formulation #50 (12.5% propylene glycol, 5% Dextran 40, 3% glucose), and Formulation #51 (12.5% propylene glycol, 5% Dextran 40, 0.5% glucose). Data points represent individual measurements with bars indicating mean values. Statistical significance is indicated by asterisk (*), “ns” indicates not significant.

[00105] **FIGS. 9A-9B** show exemplary experimental results comparing different propylene glycol (PG) concentrations in cell cryopreservation and delivery solutions. **FIG. 9A** shows post-thaw cell viability percentages (%) measured at 0 and 6 hours after thawing for formulations containing 8% or 10% PG combined with 1% Dextran 40. **FIG. 9B** shows replating efficiency percentages (%) for the same formulations, with sample sizes indicated (n=2 for T0; n=1 for T6). Data are presented as mean values with error bars indicating standard deviation.

[00106] **FIGS. 10A-10B** show exemplary experimental results evaluating the impact of propylene glycol (PG) concentration in exemplary cell cryopreservation and delivery solutions. **FIG. 10A** shows cell viability percentages (%) and **FIG. 10B** shows replating efficiency percentages (%) comparing 10% versus 12.5% PG in Base A (1% Dextran 40, 3% glucose) and Base B (5% Dextran 40, 3% glucose). Data points represent individual measurements with bars indicating mean values.

[00107] **FIGS. 11A-11B** show exemplary experimental results evaluating the effect of Dextran 40 concentration in exemplary cell cryopreservation and delivery solutions. **FIG. 11A** shows post-thaw cell viability percentages (%) measured pre-cryopreservation, immediately after thawing (0 hour), and after 6 hours for formulations containing 12.5% propylene glycol with either 5% or 1% Dextran 40. **FIG. 11B** shows replating efficiency

percentages (%) at 0 and 6 hours post-thaw for the same formulations. Data points represent individual measurements with bars indicating mean values. Statistical significance is indicated by asterisk (*), “ns” indicates not significant.

[00108] **FIGS. 12A-12B** show exemplary experimental results comparing different propylene glycol (PG) concentrations in exemplary cell cryopreservation and delivery solutions. **FIG. 12A** shows post-thaw cell viability percentages (%) measured pre-cryopreservation, immediately after thawing (0 hour), and after 6 hours for formulations containing either 10% or 12.5% PG with 1% Dextran 40. **FIG. 12B** shows replating efficiency percentages (%) at 0 and 6 hours post-thaw for the same formulations. Data points represent individual measurements with bars indicating mean values. Statistical significance is indicated by asterisks, “ns” indicates not significant.

[00109] **FIGS. 13A-13B** show experimental results comparing different formulations of cell cryopreservation and delivery solutions. **FIG. 13A** shows post-thaw cell viability percentages (%) and **FIG. 13B** shows replating efficiency percentages (%) comparing four exemplary cell cryopreservation and delivery solutions: Formulation #47 (10% propylene glycol, 1% Dextran 40, 3% glucose), Formulation #49 (10% propylene glycol, 1% Dextran 40, 0.5% glucose), Formulation #50 (12.5% propylene glycol, 5% Dextran 40, 3% glucose), and Formulation #51 (12.5% propylene glycol, 5% Dextran 40, 0.5% glucose). Data points represent individual measurements with bars indicating mean values. Statistical significance is indicated by "ns" for not significant.

[00110] **FIGS. 14A-14C** show exemplary experimental results comparing different penetrating cryoprotectants in exemplary cell cryopreservation and delivery solutions. **FIG. 14A** shows cell viability percentages (%) and **FIG. 14B** shows replating efficiency percentages (%) comparing formulations containing either 10% propylene glycol or 10% glycerol. **FIG. 14C** shows controlled rate freezing (CRF) temperature profiles over time comparing the CRF chamber temperature (lower black line) with the temperature profiles of solutions containing 10% propylene glycol (light gray line) or 10% glycerol (dark gray line) during a 60-minute freezing process. Data points in **FIGS. 14A and 14B** represent individual measurements with bars indicating mean values.

[00111] **FIGS. 15A-15B** show exemplary experimental results evaluating different cryoprotective agents (CPA) in exemplary cell cryopreservation and delivery solutions. **FIG.**

15A shows post-thaw cell viability percentages (%) measured pre-cryopreservation, immediately after thawing (0 hour), and after 6 hours for formulations containing either 10% propylene glycol or 10% glycerol with 5% Dextran 40. **FIG. 15B** shows replating efficiency percentages (%) at 0 and 6 hours post-thaw for the same formulations. Data points represent individual measurements with bars indicating mean values.

[00112] **FIGS. 16A-16D** show exemplary experimental results comparing certain NaCl concentrations in exemplary cell cryopreservation and delivery solutions. **FIG. 16A** and **FIG. 16C** show cell viability percentages (%) for different NaCl concentrations (0.72% and 0.55% NaCl in **FIG. 16A**; 0.55%, 0.36%, and 0.6% NaCl in **FIG. 16C**). **FIG. 16B** and **FIG. 16D** show replating efficiency percentages (%) for the same NaCl concentrations. Data points represent individual measurements with bars indicating mean values.

[00113] **FIGS. 17A-17B** show exemplary experimental results comparing exemplary cell cryopreservation and delivery solutions with different osmolarities. **FIG. 17A** shows post-thaw cell viability percentages (%) measured pre-cryopreservation, immediately after thawing (0 hour), and after 6 hours, and **FIG. 17B** shows replating efficiency percentages (%) at 0 and 6 hours post-thaw. Formulations compared include 10% propylene glycol (PG) + 1% Dextran 40 (~1900 mOsm/kg) versus 10% PG + 1% Dextran 40 with reduced NaCl (~2100 mOsm/kg). Data points represent individual measurements with bars indicating mean values. Statistical significance is indicated by asterisks.

[00114] **FIGS. 18A-18B** show exemplary experimental results evaluating different NaCl concentrations in cell cryopreservation and delivery solutions. **FIG. 18A** shows post-thaw cell viability percentages (%) and **FIG. 18B** shows replating efficiency percentages (%) comparing three NaCl concentrations: Formulation 47 (10% propylene glycol, 1% Dextran 40, 3% glucose), Formulation 47A (10% propylene glycol, 1% Dextran 40, 3% glucose), and Formulation 52 (10% propylene glycol, 1% Dextran 40, 3% glucose). Data points represent individual measurements with bars indicating mean values. Statistical significance is indicated by “ns” for not significant.

[00115] **FIGS. 19A-19B** show exemplary experimental results evaluating the effects of different antioxidant additives in exemplary cell cryopreservation and delivery solutions. **FIG. 19A** shows post-thaw cell viability percentages (%) measured pre-cryopreservation, immediately after thawing (0 hour), and after 6 hours, and **FIG. 19B** shows

replating efficiency percentages (%) at 0 and 6 hours post-thaw. The exemplary cell cryopreservation and delivery solutions formulations compared include 10% propylene glycol (PG) + 5% Dextran 40 alone, with 0.15% GSH + 0.1% isoleucine, or with 5% proline, as indicated along the x-axis. Data points represent individual measurements with bars indicating mean values.

[00116] **FIGS. 20A-20B** show exemplary experimental results evaluating the effect of antioxidant additives glutathione (GSH) and isoleucine (IL) in exemplary cell cryopreservation and delivery solutions. **FIG. 20A** shows post-thaw cell viability percentages (%) measured pre-cryopreservation, immediately after thawing (0 hour), and after 6 hours, and **FIG. 20B** shows replating efficiency percentages (%) at 0 and 6 hours post-thaw. The exemplary cell cryopreservation and delivery solutions compared contain 12.5% propylene glycol (PG) + 1% Dextran 40 with and without GSH/IL additives. Data points represent individual measurements with bars indicating mean values. Statistical significance is indicated by “ns” for not significant.

[00117] **FIGS. 21A-21B** show exemplary experimental results evaluating the effect of pH in exemplary cell cryopreservation and delivery solutions. **FIG. 21A** shows post-thaw cell viability percentages (%) measured pre-cryopreservation, immediately after thawing (0 hour), and after 6 hours, and **FIG. 21B** shows replating efficiency percentages (%) at 0 and 6 hours post-thaw. The exemplary cell cryopreservation and delivery solutions tested include 10% propylene glycol (PG) + 5% Dextran 40 at either pH 7.4 or pH 7.8, as indicated. Data points represent individual measurements with bars indicating mean values.

[00118] **FIGS. 22A-22B** show schematic illustrations comparing one-step versus two-step methods for preparing cell cryopreservation and delivery solutions. **FIG. 22A** shows an exemplary one-step preparation method where cells are directly resuspended in the final cryopreservation formulation before aliquoting and controlled rate freezing (CRF). **FIG. 22B** shows an exemplary two-step preparation method where cells are first resuspended in an isotonic solution (Solution A) followed by gradual addition of a double-concentrated cell cryopreservation solution (Solution B, 2X CPAs).

[00119] **FIGS. 23A-23B** show exemplary experimental results comparing one-step versus two-step addition methods for preparing exemplary cell cryopreservation and delivery solutions. **FIG. 23A** shows post-thaw cell viability percentages (%) and **FIG. 23B** shows

replating efficiency percentages (%) for cells prepared using either one-step direct addition or two-step gradual addition of a formulation containing 10% propylene glycol, 1% Dextran 40, 3% glucose. Data points represent individual measurements with bars indicating mean values.

[00120] **FIGS. 24A-24B** show exemplary experimental results comparing one-step versus two-step addition methods for preparing an exemplary cell cryopreservation and delivery solution. **FIG. 24A** shows post-thaw cell viability percentages (%) and **FIG. 24B** shows replating efficiency percentages (%) comparing the same formulation prepared using either one-step or two-step addition methods. Data points represent individual measurements with bars indicating mean values. Statistical significance is indicated by “ns” for not significant.

[00121] **FIGS. 25A-25B** show exemplary experimental results comparing cell viability across different exemplary cryopreservation and delivery solutions. **FIG. 25A** and **FIG. 25B** show post-thaw cell viability percentages (%) for various formulations including CSI (cell suspension for injection), StemCellBanker, and different combinations of propylene glycol (PG) and Dextran 40 concentrations (10% or 12.5% PG with 1% or 5% Dextran 40). Data points represent individual measurements with bars indicating mean values.

[00122] **FIGS. 26A-26B** show exemplary experimental results comparing replating efficiency across different exemplary cryopreservation and delivery solutions. In particular, **FIG. 26A** and **FIG. 26B** show replating efficiency percentages (%) for various formulations including CSI (cell suspension for injection), StemCellBanker, and different combinations of propylene glycol (PG) and Dextran 40 concentrations (10% or 12.5% PG with 1% or 5% Dextran 40). Data points represent individual measurements with bars indicating mean values. Statistical significance is indicated by "ns" for not significant.

[00123] **FIGS. 27A-27B** show experimental results comparing cell preservation outcomes between conventional approaches and a DMSO-free formulation. **FIG. 27A** shows cell viability percentages (%) and **FIG. 27B** shows replating efficiency percentages (%) for three different approaches: a conventional approach, STEM-CELLBANKER EX, and a thaw-and-inject formulation (DMSO-free). The data demonstrate comparable cell viability and replating efficiency between the DMSO-free formulation and traditional DMSO-containing preparations. Data points represent individual measurements with bars indicating mean values.

[00124] **FIGS. 28A-28B** show exemplary experimental results comparing different exemplary cryopreservation and cell delivery solutions. **FIG. 28A** shows average cell viability percentages (%) comparing standard STEM-CELLBANKER, STEM-CELLBANKER EX, and an in-house cryopreservation and cell delivery solution (Formulation 50). **FIG. 28B** shows average live cell concentrations (cells/mL in millions) comparing STEM-CELLBANKER (SCB) EX and Formulation 50. Formulation 50 includes 12.5% propylene glycol, 5% Dextran 40. Data points represent individual measurements with bars indicating mean values.

[00125] **FIGS. 29A-29B** show exemplary gene expression data comparing on-target and off-target markers in dopaminergic neural precursor cells after cryopreservation in exemplary cryopreservation and delivery solutions. **FIG. 29A** shows expression of on-target dopaminergic neuron markers (FOXA2, OTX2, LMX1A, and EN1) and **FIG. 29B** shows expression of off-target markers (CRABP1, PAX6, CD36, and KI67) assessed by qPCR at DIV0. Cells were cryopreserved in Standard STEM-CELLBANKER, STEM-CELLBANKER EX, or Formulation 50. Expression levels are normalized to geometric mean and presented as ΔC_q values. Data points represent individual measurements.

[00126] **FIGS. 30A-30B** show exemplary gene expression data comparing on-target and off-target markers in dopaminergic cells after 5 days in vitro (DIV5) following cryopreservation in exemplary cell cryopreservation and delivery solutions. **FIG. 30A** shows expression of on-target dopaminergic neuron markers (FOXA2, OTX2, LMX1A, EN1, and TH) and **FIG. 30B** shows expression of off-target markers (CRABP1, PAX6, CD36, and KI67) assessed by qPCR. Cells were cryopreserved in STEM-CELLBANKER, STEM-CELLBANKER EX, XT-THRIVE, or Formulation 50. Expression levels are normalized to geometric mean and presented as ΔC_q values. Data points represent individual measurements.

[00127] **FIGS. 31A-31B** show exemplary experimental results evaluating replating efficiency of dopaminergic neurons (generated under 2D cell differentiation conditions) after cryopreservation in an exemplary cell cryopreservation and delivery solution. **FIG. 31A** shows replating efficiency percentages (%) with ROCK inhibitor (+Rocki) and **FIG. 31B** shows replating efficiency percentages (%) without ROCK inhibitor (-Rocki) for cells cryopreserved in Control, SCB EX, or BRT-1 formulations. Data are presented as mean values with error bars indicating standard deviation.

[00128] FIGS. 32A-32B show exemplary experimental results evaluating replating efficiency of dopaminergic neurons (generated under 3D cell differentiation conditions) after cryopreservation in an exemplary cell cryopreservation and delivery solution. FIG. 32A shows replating efficiency percentages (%) with ROCK inhibitor (+Rocki) and FIG. 32B shows replating efficiency percentages (%) without ROCK inhibitor (-Rocki) for cells cryopreserved in SCB, SCB EX, or F50 formulations. Data are presented as mean values with error bars indicating standard deviation.

DETAILED DESCRIPTION

[00129] Aspects of the present disclosure relate to cell cryopreservation and delivery solutions, as well as cell suspensions including the cell cryopreservation and delivery solutions, and methods of preparing and treating a subject with said cell suspensions. In particular, the cell cryopreservation and delivery solutions of the disclosure allow for a cell suspension to be thawed and directly administered to a subject after cryopreservation.

[00130] Current cell therapies require cryopreservation of cells in a cryopreservation solution for long term storage in liquid nitrogen vapor. To deliver the cells, reformulation of the cells in a suitable delivery vehicle for transplantation is often necessary as conventional cryopreservation solutions are not suitable for injection due to toxicity concerns.

[00131] Reformulation requires cells to be washed of previous cryoprotectant and resuspended in a delivery solution to make the cell dose. This just-in-time manufacturing process is arduous, time consuming and requires skilled personnel or a specialized device to complete. Therefore, the development of a solution that can simultaneously act as a cryopreservation solution, as well as a suitable and safe solution for injection can expedite cell administration, reducing the need for specialized devices or personnel for the preparation of the cell dose. Additionally, the new cryopreservation solution and cell delivery solution would likely increase clinical adoption due to ease of use.

[00132] Herein we describe the cell cryopreservation and delivery solutions that are intended for injection. The solutions described herein enables efficient cryopreservation of cells, e.g., dopaminergic neuronal precursor cells, comparable to currently used

cryopreservation solutions, is composed of USP grade ingredients that is safe for injection, can keep cells healthy during the brief period between thaw and administration, and, in some embodiments, keep cells in suspension (aiding in dose uniformity for administration) as a secondary objective.

[00133] In some embodiments, the cell cryopreservation and delivery solutions described herein are composed of a cell permeable cryoprotectant agent (CPA) (e.g., dimethylsulfoxide (DMSO) or propylene glycol), a stabilizer, such as, a cell impermeable CPA (e.g., dextrans, polysaccharides, sugars), salts (Na, K, Cl, PO₄, Mg, etc), a pH buffering system (HEPES, phosphates, Good's buffering agents etc.), ROS scavengers, etc. within an aqueous system. In some embodiments, the cell cryopreservation and delivery solution comprises one or more antioxidants. The formulation enables the cells to be effectively cryopreserved while simultaneously being suitable for injection. Pharmaceutical grade excipients can be used to ensure safe injection.

[00134] In some embodiments, the disclosure provides methods of thawing and administering a cell solution to a subject, wherein the method does not require washing or diluting the cells prior to administration. The method comprises thawing a cryopreserved cell solution and directly administering the thawed cells to a subject. In some embodiments, the cryopreservation solution comprises a commercially available cryopreservation solution. In some embodiments, the commercially available solution is manufactured by Zenogen Pharma Co., Ltd., and sold under the trade name STEMCELL BANKER. In some embodiments, the solution comprises STEMCELL BANKER EX. In some embodiments, the solution comprises STEMCELL BANKER DMSO Free. In some embodiments, the solution comprises a cryopreservation solution that is manufactured by Akron Biotech and sold under the trade name CryoNovo P24. In some embodiments, the solution comprises a cryopreservation solution that is manufactured by BioVerde and sold under the trade name CryoScarless DMSO Free. In some embodiments, the solution comprises a cryopreservation solution manufactured by Irvine Scientific and sold under the trade name Prime-XV Stem FreezeIS DMSO-Free.

[00135] In some embodiments, this disclosure provides a cell cryopreservation and delivery solution for cryopreserving and delivering cells to a subject in need thereof. In some embodiments, the cryopreservation and delivery solution comprises propylene glycol as a cryoprotectant. In some such embodiments, the propylene glycol is present at a concentration

of about 10% wt. In some embodiments, the cryopreservation and delivery solution comprises dextran (e.g., Dextran 40). In some such embodiments, the dextran is present at a concentration of about 1% wt. In some embodiments, the cryopreservation and delivery solution comprises glucose (e.g., dextrose). In some such embodiments, the glucose is present at a concentration of about 3% wt. In some embodiments, the cryopreservation and delivery solution comprises sodium chloride. In some such embodiments, the sodium chloride is present at a concentration of about 0.36% wt. In some embodiments, the cryopreservation and delivery solution comprises HEPES buffer with a pH of about 7.4 ± 0.1 . In some embodiments, the cryopreservation and delivery solution further comprises potassium chloride, magnesium chloride, and calcium chloride.

[00136] In some embodiments, this disclosure provides a cell cryopreservation and delivery solution for cryopreserving and delivering cells to a subject in need thereof. In some embodiments, the cryopreservation and delivery solution comprises propylene glycol as a cryoprotectant. In some such embodiments, the propylene glycol is present at a concentration of about 10% wt. In some embodiments, the cryopreservation and delivery solution comprises dextran (e.g., Dextran 40). In some such embodiments, the dextran is present at a concentration of about 1% wt. In some embodiments, the cryopreservation and delivery solution comprises glucose (e.g., dextrose). In some such embodiments, the glucose is present at a concentration of about 0.5% wt. In some embodiments, the cryopreservation and delivery solution comprises sodium chloride. In some such embodiments, the sodium chloride is present at a concentration of about 0.36% wt. In some embodiments, the cryopreservation and delivery solution comprises HEPES buffer with a pH of about 7.4 ± 0.1 . In some embodiments, the cryopreservation and delivery solution further comprises potassium chloride, magnesium chloride, and calcium chloride.

[00137] In some embodiments, this disclosure provides a cell cryopreservation and delivery solution for cryopreserving and delivering cells to a subject in need thereof. In some embodiments, the cryopreservation and delivery solution comprises propylene glycol as a cryoprotectant. In some such embodiments, the propylene glycol is present at a concentration of about 12.5% wt. In some embodiments, the cryopreservation and delivery solution comprises dextran (e.g., Dextran 40). In some such embodiments, the dextran is present at a concentration of about 5% wt. In some embodiments, the cryopreservation and delivery solution comprises glucose (e.g., dextrose). In some such embodiments, the glucose is present

at a concentration of about 3% wt. In some embodiments, the cryopreservation and delivery solution comprises sodium chloride. In some such embodiments, the sodium chloride is present at a concentration of about 0.36% wt. In some embodiments, the cryopreservation and delivery solution comprises HEPES buffer with a pH of about 7.4 ± 0.1 . In some embodiments, the cryopreservation and delivery solution further comprises potassium chloride, magnesium chloride, and calcium chloride.

[00138] In one some embodiments, this disclosure provides a cell cryopreservation and delivery solution for cryopreserving and delivering cells to a subject in need thereof. In some embodiments, the cryopreservation and delivery solution comprises propylene glycol as a cryoprotectant. In some such embodiments, the propylene glycol is present at a concentration of about 12.5% wt. In some embodiments, the cryopreservation and delivery solution comprises dextran (e.g., Dextran 40). In some such embodiments, the dextran is present at a concentration of about 5% wt. In some embodiments, the cryopreservation and delivery solution comprises glucose (e.g., dextrose). In some such embodiments, the glucose is present at a concentration of about 0.5% wt. In some embodiments, the cryopreservation and delivery solution comprises sodium chloride. In some such embodiments, the sodium chloride is present at a concentration of about 0.36% wt. In some embodiments, the cryopreservation and delivery solution comprises HEPES buffer with a pH of about 7.4 ± 0.1 . In some embodiments, the cryopreservation and delivery solution further comprises potassium chloride, magnesium chloride, and calcium chloride.

[00139] Although the disclosure describes various exemplary alternatives and implementations as provided herein, it should be understood that the various features, aspects, and functionality described in one or more of the individual alternatives are not limited in their applicability to the particular alternative with which they are described. Instead, they can be applied alone or in various combinations to one or more of the other alternatives of the disclosure, whether the alternatives are described or whether the features are presented as a part of the described alternative. The breadth and scope of the present disclosure should not be limited by any exemplary alternatives described or shown herein.

I. Definitions

[00140] The following definitions supplement those in the art and are directed to the present disclosure only. The following definitions are not to be imputed to any related or unrelated case, *e.g.*, to any commonly owned patent or patent application. Although some methods and materials similar or equivalent to those described herein can be used to practice features of the disclosure, some preferred materials and methods are described herein. Accordingly, the terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting.

[00141] Unless otherwise defined herein, scientific and technical terms used in connection with the present application shall have the meanings that are commonly understood by those of ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular.

[00142] It should be understood that this invention is not limited to the particular methodology, protocols, and reagents, etc., described herein and as such may vary. The terminology used herein is for the purpose of describing particular embodiments only and is not intended to limit the scope of the present invention, which is defined solely by the claims.

[00143] As used herein, the articles “a,” “an,” and “the” are used herein to refer to one or to more than one (i.e., to at least one) of the grammatical object of the article. By way of example, “an element” means one element or more than one element.

[00144] The use of the alternative (*e.g.*, “or”) should be understood to mean either one, both, or any combination thereof of the alternatives.

[00145] As used herein, the term “about” or “approximately” refers to a quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length that varies by as much as 15%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2% or 1% compared to a reference quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length. In some instances, the term “about” or “approximately” refers a range of quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length $\pm 15\%$, $\pm 10\%$, $\pm 9\%$, $\pm 8\%$, $\pm 7\%$, $\pm 6\%$, $\pm 5\%$, $\pm 4\%$, $\pm 3\%$, $\pm 2\%$, or $\pm 1\%$ of a reference quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length.

[00146] As used herein, the terms “administration,” “administering” and variants thereof refer to the introduction of a composition or therapeutic agent (*e.g.*, a population of cells) into a subject. Administration includes concurrent and sequential introduction of the composition or therapeutic agent. Administration of the composition or therapeutic agent (*e.g.*, a population of cells) into a subject is by any suitable route, including orally, pulmonarily, intranasally, parenterally (intravenously, intramuscularly, intraperitoneally, or subcutaneously), rectally, intralymphatically, or topically. A suitable route of administration allows the composition or the agent to perform its intended function. Administration also includes self-administration and the administration by another. The administration can also be performed systemic, or it can be local. For instance, a composition or therapeutic agent (*e.g.*, a population of cells) can be administered locally, *e.g.*, by local injection into a tissue.

[00147] As used herein, the term “and/or” should be understood to mean either one, or both of, or any combination of the alternatives.

[00148] As used herein, the term “cells” can refer to any one or more types of cells noted in this or any other paragraph of the disclosure, or substitutes and/or equivalents thereof. For instance, the cells can include neural cells, myeloid cells, T cell (*e.g.*, regulatory T cells, microglial cells, or cardiac cells). The cells can also include stem cells, such as embryonic stem cells or induced pluripotent stem cells. Exemplary cells include, but are not limited to, mesenchymal stem cells, hematopoietic stem cells, embryonic stem cells or induced pluripotent stem cells, red blood cells, platelets, chondrocytes, skin cells, immune cells (*e.g.* tumor infiltrating lymphocytes, viral reconstitution T cells, dendritic cells, regulatory T cells, macrophages), neural crest stem cells, neurons, glia, smooth muscle, cardiac tissue, chondrocytes, osteocytes, glial restricted progenitors, astrocytes, oligodendrocytes, neuroblast cells, megakaryoblasts, megakaryocytes, monoblasts, monocytes, macrophages, myeloid cells, myeloid dendritic cells, microglial cells, differentiated microglial cells, microglial progenitor cells, proerythroblasts, erythroblasts, normoblasts, reticulocytes, thrombocytes, myeloblasts, progranulocytes, neutrophilic myelocytes, neutrophilic band cells, neutrophils, eosinophilic myelocytes, eosinophilic band cells, eosinophils, basophilic myelocytes, basophilic band cells, basophils, committed lymphoid progenitors, pre-NK cells, NK lymphoblasts, NK cells, thymocytes, T-lymphoblasts, T-cells, plasmacytoid dendritic cells, pre-B cells, B-lymphoblasts, B cells, plasma cells, osteoblasts, chondrocytes, myoblasts, myotubes, fibroblasts, adipocytes,

mesoderm, ectoderms, cardiomyocytes, fibroblasts, endothelial cells, pericytes, smooth muscle cells, mesothelial cells, primordial germ cells, sperm, eggs, or any other suitable type of cell. In some instances, the cells are dopaminergic neurons. Exemplary dopaminergic neurons include midbrain dopaminergic neurons, authentic midbrain dopaminergic neurons, midbrain dopaminergic neuron progenitor cells, dopaminergic neuron progenitor cells, and dopaminergic neuron precursor cells.

[00149] As used herein, the term “cell cryopreservation and delivery solution” is any solution that is added to a container containing cells so that the cells can be cryopreserved, thawed and administered to a subject. The cell cryopreservation and delivery solution can be used to prepare a cell suspension for cryopreservation and administration following thawing of cells in the cell cryopreservation and delivery solution.

[00150] As used herein, the term “cell suspension for cryopreservation and administration” refers to the cell cryopreservation and delivery solution in combination with one or more cells, with one or more optional, additional components, that can be administered to a subject.

[00151] As used herein, the term “cell wash solution” is any solution that is added to a container containing cells and an amount of cryoprotectant after removal of that container from a below 0°C environment, wherein the solution allows for the removal or dilution of at least part of the cryoprotectant.

[00152] As used herein, the term “cryoprotectant” refers to a cell permeable substance that is used to reduce or eliminate cell damage caused by freezing and thawing processes that are inevitably accompanied by ice crystal formation and ionic and osmotic imbalance when cells and/or tissues are preserved at temperatures below 0°C. The cryoprotectant is not limited to a certain substance, as long as it is able to reduce cell damage during below 0°C preservation. Nonlimiting examples thereof include a cell permeating-type cryoprotectant, such as dimethyl sulfoxide (DMSO), glycerol, propylene glycol, ethylene glycol, etc.

[00153] As used herein, the term “energy source” refers to any component that can provide chemical energy to one or more cells.

[00154] As used herein, the term “Parkinson’s disease” refers to a neurodegenerative disorder characterized by extensive degeneration of dopaminergic neurons in the substantia nigra region in the brain. Parkinson’s disease manifest in changes of both motor and non-motor functions. Exemplary changes in motor functions that are symptomatic of Parkinson’s disease include, but are not limited to, tremor, hypokinesia, postural instability, abnormal gait and swallowing disturbances. Exemplary changes in non-motor functions that are symptomatic of Parkinson’s disease include, but are not limited to, autonomic and neuropsychiatric disturbances such as anosmia or sleep abnormalities. Parkinson’s disease can be characterized as early Parkinson’s disease, or it can be characterized as advanced Parkinson’s disease, with more severe symptoms in the more advanced stages of the disease.

[00155] As used herein, the terms “patient,” “subject,” “individual,” and the like are used interchangeably and refer to any animal, or cells thereof, whether in vitro or in situ, amenable to the compositions and methods described herein. In some instances, the patient, subject or individual is a human.

[00156] As used herein, the term “pH buffer” refers to any component that can be used to maintain a constant pH in a solution.

[00157] As used herein, the term “pH indicator” refers to any component or substance that changes its properties in response to a change in pH. Such changes in properties include a change in optical properties, such as a color change.

[00158] As used herein, the term “stabilizing agent” refers to any component that can act to reduce or prevent degradation of other solution components. Without limitation, a stabilizing agent includes non-permeating-type cryoprotectants, such as sucrose, carboxymethylcellulose salts, carboxymethylcellulose (CMC), monosaccharide, disaccharide, etc.

[00159] As used herein, the term “substantially” or “essentially” refers to a quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length that is about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% or higher compared to a reference quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length. In some instances, the terms “essentially the same” or “substantially the same” refer a range of quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length that is about the same as a reference

quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length.

[00160] As used herein, the term “treat,” or a grammatical equivalent thereof, refers to a means to reduce the frequency or severity of at least one sign or symptom of a disease or disorder experienced by a subject.

[00161] As used herein the term “vitamin” includes any of various fat-soluble or water-soluble organic substances that are important in very small quantities to the nutrition of most animal cells and do not provide energy or serve as building units. Non-limiting examples include vitamin A, Vitamin B1 (thiamine), Vitamin B2 (riboflavin), Vitamin B3 (niacin or niacinamide), Vitamin B5 (pantothenic acid), Vitamin B6 (pyridoxine, pyridoxal, or pyridoxamine, or pyridoxine hydrochloride), Vitamin B7 (biotin), Vitamin B9 (folic acid), and Vitamin B12 (various cobalamins; commonly cyanocobalamin in vitamin supplements), vitamin C, vitamin D, vitamin E, vitamin K, K1 and K2 (i.e. MK-4, MK-7), folic acid and biotin) either obtained naturally from plant and animal foods or synthetically made, as well as pro-vitamins, derivatives, and/or analogs thereof.

[00162] Throughout this disclosure, various aspects of the invention can be presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the invention. Accordingly, the description of a range should be considered to have specifically disclosed all the possible subranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed subranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 2.7, 3, 4, 5, 5.3, and 6. This applies regardless of the breadth of the range.

II. Cell Cryopreservation and Delivery Solutions

[00163] Certain aspects of the disclosure provide cell cryopreservation and delivery solutions. In some embodiments, the cell cryopreservation and delivery solution

includes one or more energy source components, one or more salts, and one or more cryoprotectants.

[00164] In some embodiments, the cell cryopreservation and delivery solution includes about 0.5 mM to about 280 mM of the one or more energy source components. In some embodiments, the cell cryopreservation and delivery solution includes about 1 mM to about 270 mM, about 2 mM to about 260 mM, about 5 mM to about 250 mM, about 10 mM to about 240 mM, about 20 mM to about 230 mM, about 30 mM to about 220 mM, about 40 mM to about 210 mM, about 50 mM to about 200 mM, about 60 mM to about 190 mM, about 70 mM to about 180 mM, about 80 mM to about 170 mM, about 90 mM to about 160 mM, or about 100 mM to about 150 mM of the one or more energy source components. In some embodiments, the cell cryopreservation and delivery solution includes about 0.5 mM to about 20 mM, about 20 mM to about 40 mM, about 40 mM to about 60 mM, about 60 mM to about 80 mM, about 80 mM to about 100 mM, about 100 mM to about 120 mM, about 120 mM to about 140 mM, about 140 mM to about 160 mM, about 160 mM to about 180 mM, about 180 mM to about 200 mM, about 200 mM to about 220 mM, about 220 mM to about 240 mM, about 240 mM to about 260 mM, or about 260 mM to about 280 mM of the one or more energy source components. In some embodiments, the cell cryopreservation and delivery solution includes about 145 mM to about 165 mM of the one or more energy source components. In some embodiments, the cell cryopreservation and delivery solution includes about 10 mM to about 50 mM of the one or more energy source components. In some embodiments, the cell cryopreservation and delivery solution includes about 25 mM to about 180 mM of the one or more energy source components. In some embodiments, the cell cryopreservation and delivery solution includes about 20 mM to about 30 mM of the one or more energy source components. In some embodiments, the cell cryopreservation and delivery solution includes about 15 mM to about 25 mM of the one or more energy source components. In some embodiments, the cell cryopreservation and delivery solution includes about 22.1 mM of the one or more energy source. In some embodiments, the cell cryopreservation and delivery solution includes about 24.6 mM of the one or more energy source. In some embodiments, the cell cryopreservation and delivery solution includes about 26 mM of the one or more energy source. The one or more energy source components of the cell cryopreservation and delivery solution can include any suitable sugar, such as dextrose, fructose, galactose, glucose, lactose, maltose, sucrose. In some embodiments, the one or more energy source components include dextrose or glucose. In some embodiments, the cell

cryopreservation and delivery solution has dextrose as the energy source component. In some embodiments, the cell cryopreservation and delivery solution has glucose as the energy source component.

[00165] In some embodiments, the cell cryopreservation and delivery solution includes about 0.1 mM to about 200 mM of each of the one or more salts. In some embodiments, the cell cryopreservation and delivery solution includes about 0.1 mM to about 200 mM, about 1 mM to about 190 mM, about 1 mM to about 180 mM, about 5 mM to about 170 mM, about 10 mM to about 160 mM, about 15 mM to about 150 mM, about 20 mM to about 140 mM, about 25 mM to about 130 mM, about 30 mM to about 120 mM, about 35 mM to about 110 mM, about 40 mM to about 100 mM, about 50 mM to about 90 mM, or about 60 mM to about 1 mM of each of the one or more salts. In some embodiments, the cell cryopreservation and delivery solution includes about 0.1 mM to about 10 mM, about 0.5 mM to about 20 mM, about 20 mM to about 40 mM, about 40 mM to about 60 mM, about 60 mM to about 80 mM, about 80 mM to about 100 mM, about 100 mM to about 120 mM, about 120 mM to about 140 mM, about 140 mM to about 160 mM, about 160 mM to about 180 mM, or about 180 mM to about 200 mM of each of the one or more salts. In some embodiments, the cell cryopreservation and delivery solution includes about 10 mM to about 200 mM of each of the one or more salts. In some embodiments, the one or more salts includes one or more of calcium chloride, magnesium chloride, potassium chloride, sodium chloride and sodium phosphate. In some embodiments, the cell cryopreservation and delivery solution includes a combination of calcium chloride, magnesium chloride, potassium chloride, sodium chloride and sodium phosphate, each independently having a concentration described herein.

[00166] In some embodiments, the cell cryopreservation solution includes about 0.1 mM to about 2.5 mM of calcium chloride. In some embodiments, the cell cryopreservation solution includes about 0.1 mM to about 0.5 mM, 0.5 mM to about 1.0 mM, 1.0 mM to about 1.2 mM, about 1.2 mM to about 1.4 mM, about 1.4 mM to about 1.6 mM, about 1.6 mM to about 1.8 mM, about 1.8 mM to about 2.0 mM, about 2.0 mM to about 2.2 mM, about 2.2 mM to about 2.4 mM, or about 2.3 mM to about 2.5 mM of calcium chloride. In some embodiments, the cell cryopreservation and delivery solution includes about 1.6 mM of calcium chloride. In some embodiments, the cell cryopreservation and delivery solution includes about 1.6 mM of calcium chloride. In some embodiments, the cell cryopreservation

and delivery solution includes about 1.8 mM of calcium chloride. In some embodiments, the cell cryopreservation and delivery solution includes about 2.0 mM of calcium chloride as a salt at a concentration described herein.

[00167] In some embodiments, the cell cryopreservation solution includes about 0.1 mM to about 1.5 mM of magnesium chloride. In some embodiments, the cell cryopreservation solution includes about 0.1 mM to about 0.3 mM, about 0.3 mM to about 0.5 mM, about 0.5 mM to about 0.7 mM, about 0.7 mM to about 0.9 mM, about 0.9 mM to about 1.1 mM, about 1.1 mM to about 1.3 mM, or about 1.3 mM to about 1.5 mM of magnesium chloride. In some embodiments, the cell cryopreservation and delivery solution includes about 0.7 mM of magnesium chloride. In some embodiments, the cell cryopreservation and delivery solution includes about 0.7 mM of magnesium chloride. In some embodiments, the cell cryopreservation and delivery solution includes about 0.8 mM of magnesium chloride. In some embodiments, the cell cryopreservation and delivery solution includes about 0.9 mM of magnesium chloride as a salt at a concentration described herein.

[00168] In some embodiments, the cell cryopreservation solution includes about 4.0 mM to about 6.5 mM potassium chloride. In some embodiments, the cell cryopreservation solution includes about 4.0 mM to about 4.5 mM, 4.5 mM to about 4.7 mM, about 4.7 mM to about 4.9 mM, about 4.9 mM to about 5.1 mM, about 5.1 mM to about 5.3 mM, about 5.3 mM to about 5.5 mM, about 5.5 mM to about 5.7 mM, about 5.7 mM to about 5.9 mM, about 5.9 mM to about 6.1 mM, about 6.1 mM to about 6.3 mM, or about 6.3 mM to about 6.5 mM of potassium chloride. In some embodiments, the cell cryopreservation and delivery solution includes about 4.8 mM potassium chloride. In some embodiments, the cell cryopreservation and delivery solution includes about 5.3 mM potassium chloride. In some embodiments, the cell cryopreservation and delivery solution includes about 5.8 mM potassium chloride as a salt at a concentration described herein.

[00169] In some embodiments, the cell cryopreservation solution includes about 20 mM to about 150 mM sodium chloride. In some embodiments, the cell cryopreservation solution includes about 20 mM to about 40 mM, 40 mM to about 60 mM, about 60 mM to about 80 mM, about 80 mM to about 100 mM, about 100 mM to about 120 mM, about 120 mM to about 140 mM, or about 130 mM to about 150 mM sodium chloride. In some embodiments, the cell cryopreservation and delivery solution includes about 74.8 mM sodium chloride. In some embodiments, the cell cryopreservation and delivery solution

includes about 75 mM sodium chloride. In some embodiments, the cell cryopreservation and delivery solution includes about 83.1 mM sodium chloride. In some embodiments, the cell cryopreservation and delivery solution includes about 91.4 mM sodium chloride as a salt at a concentration described herein.

[00170] In some embodiments, the cell cryopreservation solution includes about 0.1 mM to about 1.5 mM sodium phosphate monobasic. In some embodiments, the cell cryopreservation solution includes about 0.1 mM to about 0.2 mM, about 0.2 mM to about 0.4 mM, about 0.4 mM to about 0.6 mM, about 0.6 mM to about 0.8 mM, about 0.8 mM to about 1.0 mM, about 1.0 mM to about 1.2 mM, about 1.2 mM to about 1.4 mM, or about 1.3 mM to about 1.5 mM of sodium phosphate monobasic. In some embodiments, the cell cryopreservation and delivery solution includes about 0.8 mM sodium phosphate monobasic. In some embodiments, the cell cryopreservation and delivery solution includes about 0.81 mM sodium phosphate monobasic. In some embodiments, the cell cryopreservation and delivery solution includes about 0.89 mM sodium phosphate monobasic. In some embodiments, the cell cryopreservation and delivery solution includes about 0.98 mM sodium phosphate monobasic as a salt at a concentration described herein.

[00171] In some embodiments, the cell cryopreservation and delivery solution includes about 5% weight by volume (wt) to about 20% wt of the one or more cryoprotectants. In some embodiments, the cell cryopreservation and delivery solution includes about 5% wt to about 7% wt, about 7% wt to about 9% wt, about 9% wt to about 11% wt, about 11% wt to about 13% wt, about 13% wt to about 15% wt, about 15% wt to about 17% wt, about 17% wt to about 19% wt, or about 18% wt to about 20% wt of the one or more cryoprotectants. In some embodiments, the cell cryopreservation and delivery solution includes about 1% wt to about 30% wt of the one or more cryoprotectants. In some embodiments, the cell cryopreservation and delivery solution includes about 5% wt of the one or more cryoprotectants. In some embodiments, the cell cryopreservation and delivery solution includes about 10% wt of the one or more cryoprotectants. In some embodiments, the cell cryopreservation and delivery solution includes about 12.5% of the one or more cryoprotectants. In some embodiments, the cell cryopreservation and delivery solution includes about 15% wt of the one or more cryoprotectants. In some embodiments, one or more cryoprotectants consists essentially of DMSO at a wt described herein. In some

embodiments, one or more cryoprotectants consists essentially of propylene glycol at a wt described herein.

[00172] In some embodiments, the cell cryopreservation and delivery solution further includes one or more pH buffers. In some embodiments, the cell cryopreservation and delivery solution includes about 5 mM to about 50 mM of the one or more pH buffers. In some embodiments, the cell cryopreservation and delivery solution includes about 5 mM to about 40 mM of the one or more pH buffers. In some embodiments, the cell cryopreservation and delivery solution includes about 5 mM to about 10 mM, about 10 mM to about 15 mM, about 15 mM to about 20 mM, about 20 mM to about 25 mM, about 25 mM to about 30 mM, about 30 mM to about 35 mM, about 35 mM to about 40 mM, about 40 mM to about 45 mM, or about 45 mM to about 50 mM of the one or more pH buffers. In some embodiments, the cell cryopreservation and delivery solution includes about 5 mM to about 15 mM of the one or more pH buffers. In some embodiments, the cell cryopreservation and delivery solution includes about 5 mM to about 10 mM of the one or more pH buffers. In some embodiments, the cell cryopreservation and delivery solution includes about 10 mM to about 15 mM of the one or more pH buffers. In some embodiments, the cell cryopreservation and delivery solution includes about 9.7 mM of the one or more pH buffers. In some embodiments, the cell cryopreservation and delivery solution includes about 10.8 mM of the one or more pH buffers. In some embodiments, the cell cryopreservation and delivery solution includes about 11.9 mM of the one or more pH buffers. The one or more pH buffers of the cell cryopreservation and delivery solution can include any suitable buffering agent, such as a zwitterionic organic chemical buffering agent, examples of which include, but are not limited to, 4-(2-hydroxyethyl)-1- piperazineethanesulfonic acid (HEPES), sodium bicarbonate, 4-Morpholinepropanesulfonic acid, 3-(N-morpholino)propanesulfonic acid (MOPS), and 2-(N-morpholino)ethanesulfonic acid (MES). In some embodiments, the cell cryopreservation and delivery solution includes HEPES, a phosphate, or a combination thereof as pH buffers. In some embodiments, the pH buffer is HEPES. In some embodiments, the pH buffer is a phosphate.

[00173] In some embodiments, the cell cryopreservation and delivery solution further includes one or more stabilizing agents. In some embodiments, the one or more stabilizing agents include a non-permeable type cryoprotectant. In some embodiments, the cell cryopreservation and delivery solution includes about 0.01% wt to about 25% wt of the

one or more stabilizing agents. In some embodiments, the cell cryopreservation and delivery solution includes about 0.01% wt to about 1% wt, 1% wt to about 5% wt, 5% wt to about 7% wt, about 7% wt to about 9% wt, about 9% wt to about 11% wt, about 11% wt to about 13% wt, about 13% wt to about 15% wt, about 15% wt to about 17% wt, about 17% wt to about 19% wt, about 18% wt to about 20% wt, about 20% wt to about 23% wt, or about 23% wt to about 25% wt of the one or more stabilizing agents. The one or more stabilizing agents of the cell cryopreservation and delivery solution can include any suitable stabilizing agent.

Exemplary stabilizing agents include, but are not limited to, albumins, dextran (including Dextran 40, as one example), poloxamer (including Poloxamer 188 as one example), an antioxidant (including reduced glutathione as one example), polyethylene glycol, carboxymethyl cellulose, hyaluronic acid, starches, acrylates, methacrylates, polyvinyl alcohols, polyethylene oxides, polypropylene oxides, polyacrylates, polyvinylpyrrolidone, polymethacrylate, poly lactic- co-glycolic acids, polyacrylamides, polylactides, chitosans, gums, guar gums, xanthan gums, carrageenans, hydroxypropyl cellulose, hydroxypropyl methyl cellulose, hydroxyethyl cellulose, cyclodextrin derivatives, beta-cyclodextrin derivatives, alginates, calcium alginates, antioxidants, and stearates. In some embodiments, the one or more stabilizing agents includes Dextran, a Poloxamer, one or more antioxidants or albumin. In some embodiments, the stabilizing agent includes dextran, a poloxamer or albumin. In some embodiments, the stabilizing agent includes dextran, or polyvinylpyrrolidone. In some embodiments, the stabilizing agent includes dextran and a poloxamer. In some embodiments, the cell cryopreservation and delivery solution includes dextran, a poloxamer, and albumin as stabilizing agents at a wt described herein.

[00174] In some embodiments, the cell cryopreservation solution includes about 0.1% wt to about 20% wt of Dextran. In some embodiments, the cell cryopreservation and delivery solution includes about 0.1% wt to about 1% wt, 1% wt to about 5% wt, 5% wt to about 7% wt, about 7% wt to about 9% wt, about 9% wt to about 11% wt, about 11% wt to about 13% wt, about 13% wt to about 15% wt, about 15% wt to about 17% wt, about 17% wt to about 19% wt, or about 18% wt to about 20% wt of Dextran. In some embodiments, the cell cryopreservation and delivery solution includes about 15.6% wt Dextran. In some embodiments, the cell cryopreservation and delivery solution includes about 15.9% wt Dextran. In some embodiments, the cell cryopreservation and delivery solution includes about 17.3% wt Dextran. In some embodiments, the cell cryopreservation and delivery solution includes about 19 % wt Dextran.

[00175] In some embodiments, the cell cryopreservation solution includes about 0.01% wt to about 20% wt of the Poloxamer. In some embodiments, the cell cryopreservation and delivery solution includes about 0.01% wt to about 1% wt, about 1% wt to about 2% wt, about 2% wt to about 5% wt, about 5% wt to about 10% wt, about 10% wt to about 15% wt, or about 15% wt to about 20% wt of the Poloxamer. In some embodiments, the cell cryopreservation and delivery solution includes about 0.07% wt of the Poloxamer. In some embodiments, the cell cryopreservation and delivery solution includes about 0.076% wt of the Poloxamer. In some embodiments, the cell cryopreservation and delivery solution includes about 0.082% wt of the Poloxamer. In some embodiments, the cell cryopreservation and delivery solution includes about 0.09% wt of the Poloxamer. In some embodiments, the Poloxamer is Poloxamer 188.

[00176] In some embodiments, the cell cryopreservation solution includes about 0.05% wt to about 0.5% wt albumin (*e.g.*, recombinant human serum albumin). In some embodiments, the cell cryopreservation solution includes about 0.05% wt to about 0.1% wt, 0.07% wt to about 0.12% wt, 0.12% wt to about 0.15% wt, 0.15% wt to about 0.18% wt, 0.18% wt to about 0.23% wt, about 0.23% wt to about 0.28% wt, about 0.28% wt to about 0.33% wt, about 0.33% wt to about 0.35% wt, about 0.35% wt to about 0.40% wt, about 0.40% wt to about 0.45% wt, or 0.45% wt to about 0.5% wt, recombinant human serum albumin. In some embodiments, the cell cryopreservation and delivery solution includes about 0.075% wt recombinant human serum albumin. In some embodiments, the cell cryopreservation and delivery solution includes about 0.083% wt recombinant human serum albumin. In some embodiments, the cell cryopreservation and delivery solution includes about 0.091% wt recombinant human serum albumin.

[00177] In some embodiments, the cell cryopreservation and delivery solution includes about 20 mM to about 30 mM dextrose; about 10 mM to about 50 mM HEPES; about 1.0 mM to about 2.5 mM calcium chloride; about 0.1 mM to about 1.5 mM magnesium chloride; about 4.5 mM to about 6.5 mM potassium chloride; about 40 mM to about 150 mM sodium chloride; about 0.1 mM to about 1.5 mM sodium phosphate monobasic; about 5% wt to about 20% wt Dextran; about 0.01% wt to about 20% wt Poloxamer; about 0.05% wt to about 0.5% recombinant human serum albumin; and about 5% wt to about 20% wt of the one or more cryoprotectants. In some embodiments, the one or more cryoprotectants consist

essentially of DMSO. In some embodiments, the one or more cryoprotectants consist essentially of propylene glycol. In some embodiments, the Poloxamer is Poloxamer 188.

[00178] In some embodiments, the cell cryopreservation and delivery solution includes about 24.6 mM dextrose; about 10.8 mM HEPES; about 1.8 mM calcium chloride; about 0.8 mM magnesium chloride; about 5.3 mM potassium chloride; about 83.1 mM sodium chloride; about 0.89 mM sodium phosphate monobasic; about 17.3% wt Dextran; about 0.082% wt Poloxamer; and about 10% wt of the one or more cryoprotectants. In some embodiments, the cell cryopreservation and delivery solution includes about 22.1 mM dextrose; about 9.7 mM HEPES; about 1.6 mM calcium chloride; about 0.7 mM magnesium chloride; about 4.7 mM potassium chloride; about 75 mM sodium chloride; about 0.8 mM sodium phosphate monobasic; about 15.9% wt Dextran; about 0.07% wt Poloxamer; and about 10% wt of the one or more cryoprotectants. In some embodiments, the one or more cryoprotectants consists essentially of DMSO. In some embodiments, the one or more cryoprotectants consists essentially of propylene glycol. In some embodiments, the Poloxamer is Poloxamer 188.

[00179] There are several components that are not included the cell cryopreservation and delivery solution (*e.g.*, a cell cryopreservation and delivery solution described herein) and can be excluded wholly or below detectable limits. Some examples of components that can be excluded are: certain components of animal origin; certain stabilizing agents, such as human serum albumin (HSA); certain salts, such as zinc sulfate, sodium bicarbonate, and ferric nitrate; certain pH indicators, such as Phenol Red; certain sources of energy, such as sodium pyruvate; certain amino acids such as Glycine, L-Alanine, L-Arginine hydrochloride, L-Asparagine-H₂O, L-Glutamine, L-Cysteine, L-Histidine hydrochloride-H₂O, L-Isoleucine, L-Leucine, L-Lysine hydrochloride, L-Methionine, L-Phenylalanine, L-Proline, L-Serine, L-Threonine, L-Tryptophan, L-Tyrosine, and L-Valine; and certain vitamins, such as Ascorbic Acid, Choline Chloride, D-Calcium pantothenate, Folic Acid, Niacinamide, Pyridoxal hydrochloride, Riboflavin, Thiamine hydrochloride, Vitamin B12 and i-Inositol. In some embodiments, the cell cryopreservation and delivery solution does not include any components of animal origin. In some embodiments, the cell cryopreservation and delivery solution does not include one or more of zinc sulfate, sodium bicarbonate, and ferric nitrate. In some embodiments, the cell cryopreservation and delivery solution does not include sodium pyruvate. In some embodiments, the cell cryopreservation and delivery

solution does not include an amino acid. In some embodiments, the cell cryopreservation and delivery solution does not include a vitamin.

[00180] In some embodiments, the cell cryopreservation and delivery solution further includes a pH indicator. In some embodiments, the pH indicator is Phenol Red.

[00181] In some embodiments, the cell cryopreservation and delivery solution comprises a pH between about 7.0 and about 8.0. In some embodiments, the cell cryopreservation and delivery solution comprises a pH between about 7.2 and about 7.8. In some embodiments, the cell cryopreservation and delivery solution comprises a pH that is less than 7.8. In some embodiments, the cell cryopreservation and delivery solution comprises a pH of about 7.4.

[00182] In some embodiments, the cell cryopreservation and delivery solution (*e.g.*, a cell cryopreservation and delivery solution described herein) further includes one or more excipients. In some embodiments, the one or more excipients include one or more of polyethylene glycol, carboxymethyl cellulose, hyaluronic acid, starches, acrylates, methacrylates, polyvinyl alcohols, polyethylene oxides, polypropylene oxides, polyacrylates, polyvinylpyrrolidone, polymethacrylate, polylactic-co-glycolic acids, polyacrylamides, polylactides, chitosans, gums, guar gums, xanthan gums, carrageenans, hydroxypropyl cellulose, hydroxypropyl methyl cellulose, hydroxyethyl cellulose, cyclodextrin derivatives, beta-cyclodextrin derivatives, alginates, calcium alginates, dextran, pentaisomaltose, hydroxyethyl starch, trehalose, sorbitol, mannitol, reduced glutathione, isoleucine, proline, ascorbic acid, N-acetyl-L-cysteine, carnitine, betaine, glycolipids, PEG lipids, lipoprotein, polysorbate 20, polysorbate 80, polyoxyl 15 hydroxy stearate, ectoine, and stearates. In some embodiments, the one or more excipients include polyvinylpyrrolidone. In some embodiments, the polyvinylpyrrolidone include one or more of polyvinylpyrrolidone K30, polyvinylpyrrolidone K12, polyvinylpyrrolidone K17, or polyvinylpyrrolidone K90. In some embodiments, the one or more excipients include hydroxyethyl starch. In some embodiments, the hydroxyethyl starch includes one or more of hydroxyethyl starch 130/0.4, hydroxyethyl starch 70/0.5, or hydroxyethyl starch 200/0.5. In some embodiments, the one or more excipients include polyethylene glycol. In some embodiments, the polyethylene glycol includes one or more of polyethylene glycol 300, polyethylene glycol 400, polyethylene glycol 600, polyethylene glycol 3350, or polyethylene glycol 4000. In some embodiments,

the one or more excipients include dextran. In some embodiments, the dextran includes one or more of dextran 5, dextran 10, dextran 20, dextran 40, or dextran 70.

[00183] In some embodiments, the cell cryopreservation and delivery solution (*e.g.*, a cell cryopreservation and delivery solution described herein) further includes an additional cryoprotectant. In some embodiments, the additional cryoprotectant is a permeating-type cryoprotectant. In some embodiments, the permeating-type cryoprotectant includes dimethyl sulfoxide (DMSO), glycerol, or propylene glycol. In some embodiments, the additional cryoprotectant is a non-permeating-type cryoprotectant. In some embodiments, the non-permeating-type cryoprotectant includes sucrose, carboxymethylcellulose salts, carboxymethylcellulose (CMC), a monosaccharide, or a disaccharide. In some embodiments, the only cryoprotectant present in the cryopreservation and delivery solution is propylene glycol.

[00184] In some embodiments, the cell cryopreservation and delivery solution (*e.g.*, a cell cryopreservation and delivery solution described herein) can be formulated to constitute various qualities, such as a particular pH, osmolarity, density or viscosity. In some embodiments, the cell cryopreservation and delivery solution is formulated to have a pH level of about 5.5 to about 9.0, or a pH level of about 6.0 to about 8.0, or a pH level of about 6.4 to about 7.8, or a pH level of about 6.8 to about 7.6, or a pH level of about 7.0 to about 7.5, a pH level of about 7.2 to about 7.4 or pH level of about 7.4 to about 7.8. In some embodiments, the cell cryopreservation and delivery solution is formulated to have an osmolarity of about 280 to about 4500 mOsm/L, or an osmolarity of about 400 to about 4000 mOsm/L, or an osmolarity of about 600 to about 3800 mOsm/L, or an osmolarity of about 1000 to about 3500 mOsm/L, or an osmolarity of about 1500 to about 3000 mOsm/L, or an osmolarity of about 2000 to about 2500 mOsm/L, or an osmolarity of about 2100 to about 2400 mOsm/L. In some embodiments, the cell cryopreservation and delivery solution is formulated to have a density of about 1.00 to about 1.08 g/mL, or a density of about 1.00 to about 1.02 g/mL, or a density of about 1.02 to about 1.04 g/mL, or a density of about 1.04 to about 1.06 g/mL, g/mL, or a density of about 1.06 to about 1.08 g/mL, or a density of about 1.08 g/mL. In some embodiments, the cell cryopreservation and delivery solution is formulated to have a relatively low viscosity, and a good cell compatibility such that both the cell cryopreservation and delivery solution is substantially not cytotoxic. The cell cryopreservation and delivery solution is configured to have a sufficient shelf life at typical or

standard storage conditions, making it ready to use for clinical applications, such as administration to a subject.

[00185] In some embodiments, the cell cryopreservation and delivery solution (*e.g.*, a cell cryopreservation and delivery solution described herein) enables cryopreservation of a plurality of cells at a therapeutically effective dose. In some embodiments, the therapeutically effective dose includes about 10^0 cells to about 10^{10} cells. In some embodiments, the therapeutically effective dose includes a range of about 10^1 cells to about 10^9 cells, about 10^2 cells to about 10^8 cells, about 10^3 cells to about 10^7 cells, about 10^4 cells to about 10^6 cells. In some embodiments, the therapeutically effective dose includes about 10^4 to about 10^{10} cells, about 10^4 to about 10^5 cells, about 10^5 to about 10^9 cells, about 10^5 to about 10^6 cells, about 10^5 to about 10^7 cells, about 10^6 to about 10^7 cells, about 10^6 to about 10^8 cells, about 10^7 to about 10^8 , from about 10^8 to about 10^9 cells, about 10^8 to about 10^{10} cells, or about 10^9 to about 10^{10} cells. In some embodiments, the therapeutically effective dose includes a range of about 10^5 cells to about 10^8 cells. In some embodiments, the therapeutically effective dose includes a range of about 10^6 cells to about 10^7 cells. In some embodiments, the therapeutically effective dose includes at least 0.25 million cells, at least 0.5 million cells, at least 0.75 million cells, at least 1 million cells, at least 1.25 million cells, at least 1.5 million cells, at least 1.75 million cells, at least 2 million cells, at least 2.25 million cells, at least 2.5 million cells, at least 2.75 million cells, at least 3 million dopaminergic, at least 3.25 million cells, at least 3.5 million cells, at least 3.75 million cells, at least 4 million cells, at least 4.5 million cells, at least 5 million cells, at least 5.5 million cells, at least 6 million cells, at least 7 million cells, at least 8 million cells, at least 9 million cells, or at least 10 million or more cells. In some embodiments, the therapeutically effective dose includes at least 1.8 million cells. In some embodiments, the therapeutically effective dose includes at least 2.7 million cells. In some embodiments, the therapeutically effective dose includes at least 5.4 million cells. In some embodiments, the plurality of cells includes differentiated midbrain dopaminergic neuronal cells.

[00186] In some embodiments, the cell cryopreservation and delivery solution (*e.g.*, a cell cryopreservation and delivery solution described herein) enables stable storage of a plurality of cells. In some embodiments, the cell cryopreservation and delivery solution enables storage of a plurality of cells for up to at least about 1 hour to 24 hours, at least about

2 hours to 18 hours, at least about 4 hours to 12 hours, or at least about 6 hours to 10 hours at 4 °C. In some embodiments, the cell cryopreservation and delivery solution enables storage of a plurality of cells for up to at least about 2 hours, at least about 4 hours, at least about 6 hours, at least about 8 hours, at least about 10 hours, or at least about 12 hours at 4 °C. In some embodiments, the cell cryopreservation and delivery solution enables storage of a plurality of cells for up to at least about 6 hours at 4 °C. In some embodiments, the plurality of cells includes differentiated midbrain dopaminergic neuronal cells.

III. Cell Suspensions

[00187] Certain aspects of the disclosure provide cell suspensions for cryopreservation and administration to a subject. In some embodiments, the cell suspension for cryopreservation and administration to a subject includes a plurality of cells, and a cell cryopreservation and delivery solution (*e.g.*, a cell cryopreservation and delivery solution described herein).

[00188] The plurality of cells can include any cell type of interest. For instance, the plurality of cells can include neural cells, myeloid cells or cardiac cells. Exemplary cell types that can be included in the plurality of cells include, but are not limited to, mesenchymal stem cells, hematopoietic stem cells, embryonic stem cells or induced pluripotent stem cells, red blood cells, platelets, chondrocytes, skin cells, immune cells (*e.g.* tumor infiltrating lymphocytes, viral reconstitution T cells, dendritic cells, regulator T cells, macrophages), neural crest stem cells, neurons, glia, smooth muscle, cardiac tissue, chondrocytes, osteocytes, glial restricted progenitors, astrocytes, oligodendrocytes, neuroblast cells, megakaryoblasts, megakaryocytes, monoblasts, monocytes, macrophages, myeloid cells, myeloid dendritic cells, microglial cells, differentiated microglial cells, microglial progenitor cells, proerythroblasts, erythroblasts, normoblasts, reticulocytes, thrombocytes, myeloblasts, progranulocytes, neutrophilic myelocytes, neutrophilic band cells, neutrophils, eosinophilic myelocytes, eosinophilic band cells, eosinophils, basophilic myelocytes, basophilic band cells, basophils, committed lymphoid progenitors, pre-NK cells, NK lymphoblasts, NK cells, thymocytes, T-lymphoblasts, T-cells, plasmacytoid dendritic cells, pre-B cells, B-lymphoblasts, B cells, plasma cells, osteoblasts, chondrocytes, myoblasts, myotubes, fibroblasts, adipocytes, mesoderm, ectoderms, cardiomyocytes, fibroblasts, endothelial cells, pericytes, smooth muscle cells, mesothelial cells, primordial germ cells,

sperm, eggs, or any progenitor or precursor thereof. The plurality of cells can also include one or more stem cells, such as pluripotent stem cells. The plurality of cells can also include non-living biologics, such as endosomes and lipid-based vesicles. In further addition to any of the cells noted above as being “cells” of this disclosure, “cells” can refer to any cell and/or tissue disclosed in U.S. Patent No. 10,280,398, U.S. Patent No. 10,711,243, International Application WO 2013/067362, and International Application WO 2021/042027, the contents of each of which are incorporated herein in their entireties.

[00189] In some instances, the plurality of cells includes neural cells. In some embodiments, the neural cells include dopaminergic neurons. In some embodiments, the dopaminergic neurons include midbrain dopaminergic neurons, authentic midbrain dopaminergic neurons, midbrain dopaminergic neuron progenitor cells, dopaminergic neuron progenitor cells, or dopaminergic neuron precursor cells. In some embodiments, the plurality of cells includes differentiated midbrain dopaminergic neuronal cells, or a precursor thereof. In some embodiments, the differentiated midbrain dopaminergic neuronal cells are differentiated midbrain dopaminergic neuron progenitor cells.

[00190] In some embodiments, the plurality of cells is derived from stem cells. In some embodiments, the stem cells are pluripotent stem cells. In some embodiments, the pluripotent stem cells are embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs), or a combination thereof. In some embodiments, the stem cells are multipotent stem cells. Non-limiting examples of stem cells that can be used to produce a plurality of cells include, but are not limited to, human, nonhuman primate or rodent nonembryonic stem cells, embryonic stem cells, induced nonembryonic pluripotent cells and engineered pluripotent cells. In some embodiments, the stem cells are human stem cells. Non-limiting examples of human stem cells include human embryonic stem cells (hESC), human pluripotent stem cell (hPSC), human induced pluripotent stem cells (hiPSC), human parthenogenetic stem cells, primordial germ cell-like pluripotent stem cells, epiblast stem cells, F-class pluripotent stem cells, somatic stem cells, cancer stem cells, or any other cell capable of lineage specific differentiation.

[00191] In some embodiments, the cell suspension for cryopreservation and administration to a subject includes a plurality of cells that is allogenic to the subject. In other embodiments, the cell suspension for cryopreservation and administration to a subject

includes a plurality of cells that is autologous to the subject. In some embodiments, the subject is a human subject. In some embodiments, the plurality of cells includes human cells.

[00192] In some embodiments, the cell suspension for cryopreservation and administration to a subject includes a plurality of cells at a range of about 10^0 cells to about 10^{10} cells. In some embodiments, the plurality of cells includes a range of about 10^1 cells to about 10^9 cells, about 10^2 cells to about 10^8 cells, about 10^3 cells to about 10^7 cells, about 10^4 cells to about 10^6 cells. In some embodiments, the plurality of cells includes about 10^4 to about 10^{10} cells, about 10^4 to about 10^5 cells, about 10^5 to about 10^9 cells, about 10^5 to about 10^6 cells, about 10^5 to about 10^7 cells, about 10^6 to about 10^7 cells, about 10^6 to about 10^8 cells, about 10^7 to about 10^8 , from about 10^8 to about 10^9 cells, about 10^8 to about 10^{10} cells, or about 10^9 to about 10^{10} cells. In some embodiments, the plurality of cells includes a range of about 10^5 cells to about 10^8 cells. In some embodiments, the plurality of cells includes a range of about 10^6 cells to about 10^8 cells. In some embodiments, the plurality of cells includes at least 0.25 million cells, at least 0.5 million cells, at least 0.75 million cells, at least 1 million cells, at least 1.25 million cells, at least 1.5 million cells, at least 1.75 million cells, at least 2 million cells, at least 2.25 million cells, at least 2.5 million cells, at least 2.75 million cells, at least 3 million dopaminergic, at least 3.25 million cells, at least 3.5 million cells, at least 3.75 million cells, at least 4 million cells, at least 4.5 million cells, at least 5 million cells, at least 5.5 million cells, at least 6 million cells, at least 7 million cells, at least 8 million cells, at least 9 million cells, or at least 10 million or more cells. In some embodiments, the plurality of cells includes at least 1.8 million cells. In some embodiments, the plurality of cells includes at least 2.7 million cells. In some embodiments, the plurality of cells includes at least 5.4 million cells. In some embodiments, the plurality of cells includes at least 7 million cells.

[00193] In some embodiments, the cell suspension for cryopreservation and administration to a subject includes a plurality of cells that has not been contacted with a cell wash buffer (*i.e.*, any solution that is used for washing the cells post thaw, for example, to remove the cryopreservation solution from cell suspension) prior to administration to the subject. Cell wash buffers are known in the art. In some embodiments, the cell wash buffer is a phosphate buffer saline (PBS), Bio-Plex Pro™ Cell Signaling Wash Buffer, or Cultrex™ 3-D Cell Wash Buffer.

[00194] In some embodiments, the cell suspension for cryopreservation and administration to a subject includes a cell cryopreservation and delivery solution that includes one or more of: one or more energy source components, one or more salts, and a one or more cryoprotectants.

[00195] In some embodiments, the cell suspension for cryopreservation and administration to a subject includes a cell cryopreservation and delivery solution that includes about 0.5 mM to about 280 mM of the one or more energy source components. In some embodiments, the cell cryopreservation and delivery solution includes about 25 mM to about 280 mM of the one or more energy source components. In some embodiments, the cell cryopreservation and delivery solution includes about 120 mM to about 190 mM of the one or more energy source components. In some embodiments, the cell cryopreservation and delivery solution includes about 145 mM to about 165 mM of the one or more energy source. In some embodiments, the cell cryopreservation and delivery solution includes about 22.1 mM of the one or more energy source. In some embodiments, the one or more energy source components include dextrose or glucose.

[00196] In some embodiments, the cell suspension for cryopreservation and administration to a subject includes a cell cryopreservation and delivery solution that about 0.1 mM to about 200 mM of each of the one or more salts. In some embodiments, the cell suspension for cryopreservation and administration to a subject includes a cell cryopreservation and delivery solution that about 10 mM to about 200 mM of each of the one or more salts. In some embodiments, the one or more salts includes one or more of calcium chloride, magnesium chloride, potassium chloride, sodium chloride and sodium phosphate or a combination thereof. In some embodiments, the cell suspension for cryopreservation and administration to a subject includes a cell cryopreservation and delivery solution that includes about 0.1 mM to about 2.5 mM of calcium chloride. In some embodiments, the cell cryopreservation and delivery solution includes about 1.6 mM of calcium chloride. In some embodiments, the cell cryopreservation and delivery solution includes about 1.8 mM of calcium chloride. In some embodiments, the cell suspension for cryopreservation and administration to a subject includes a cell cryopreservation and delivery solution that includes about 0.1 mM to about 1.5 mM of magnesium chloride. In some embodiments, the cell cryopreservation and delivery solution includes about 0.5 mM to 1.0 mM of magnesium chloride. In some embodiments, the cell cryopreservation and delivery solution includes

about 0.8 mM of magnesium chloride. In some embodiments, the cell suspension for cryopreservation and administration to a subject includes a cell cryopreservation and delivery solution that includes about 4.0 mM to about 6.5 mM potassium chloride. In some embodiments, the cell cryopreservation and delivery solution includes about 4.0 mM to 6 mM potassium chloride. In some embodiments, the cell cryopreservation and delivery solution includes about 5.3 mM potassium chloride. In some embodiments, the cell suspension for cryopreservation and administration to a subject includes a cell cryopreservation and delivery solution that includes about 20 mM to about 150 mM sodium chloride. In some embodiments, the cell cryopreservation and delivery solution includes about 60 mM to 80 mM sodium chloride. In some embodiments, the cell cryopreservation and delivery solution includes about 83.1 mM sodium chloride. In some embodiments, the cell suspension for cryopreservation and administration to a subject includes a cell cryopreservation and delivery solution that includes about 0.1 mM to about 1.5 mM sodium phosphate monobasic. In some embodiments, the cell cryopreservation and delivery solution includes about 0.8 mM sodium phosphate monobasic. In some embodiments, the cell cryopreservation and delivery solution includes about 0.89 mM sodium phosphate monobasic..

[00197] In some embodiments, the cell suspension for cryopreservation and administration to a subject includes a cell cryopreservation and delivery solution that includes about 5% weight by volume (wt) to about 25% wt of the one or more cryoprotectants. In some embodiments, the cell suspension for cryopreservation and administration to a subject includes about 1% wt to about 30% wt of the one or more cryoprotectants. In some embodiments, the cell cryopreservation and delivery solution includes about 10% wt of the one or more cryoprotectants. In some embodiments, the cell cryopreservation and delivery solution includes about 12.5 of the one or more cryoprotectants. In some embodiments, the cell cryopreservation and delivery solution includes about 15% wt of the one or more cryoprotectants. In some embodiments, the one or more cryoprotectants consists essentially of DMSO. In some embodiments, the one or more cryoprotectants consists essentially of propylene glycol.

[00198] In some embodiments, the cell suspension for cryopreservation and administration to a subject includes a cell cryopreservation and delivery solution that further includes one or more pH buffers. In some embodiments, the cell suspension for

cryopreservation and administration to a subject includes a cell cryopreservation and delivery solution that includes about 4 mM to about 40 mM of the one or more pH buffers. In some embodiments, the cell cryopreservation and delivery solution includes about 10 mM to 15 mM of the one or more pH buffers. In some embodiments, the cell cryopreservation and delivery solution includes about 10.8 mM of the one or more pH buffers. In some embodiments, the one or more pH buffers comprise 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 3-(N-morpholino)propanesulfonic acid (MOPS), 2-(N-morpholino) ethanesulfonic acid (MES), bicarbonate, or phosphates, or a combination as pH buffers.

[00199] In some embodiments, the cell suspension for cryopreservation and administration to a subject includes a cell cryopreservation and delivery solution that further includes one or more stabilizing agents. In some embodiments, the cell suspension for cryopreservation and administration to a subject includes a cell cryopreservation and delivery solution that includes about 0.01% wt to about 25% wt of the one or more stabilizing agents. In some embodiments, the one or more stabilizing agents include a non-permeable type cryoprotectant. In some embodiments, the one or more stabilizing agents includes dextran, poloxamer, one or more antioxidants, or albumin. In some embodiments, the cell suspension for cryopreservation and administration to a subject includes a cell cryopreservation and delivery solution that includes about 0.1% wt to about 20% wt of Dextran. In some embodiments, the cell cryopreservation and delivery solution includes about 0.1% wt to 10% wt. In some embodiments, the cell suspension for cryopreservation and administration to a subject includes a cell cryopreservation and delivery solution that includes about 0.01% wt to about 20% wt of the Poloxamer. In some embodiments, the cell cryopreservation and delivery solution includes about 0.07% wt of the Poloxamer. In some embodiments, the cell cryopreservation and delivery solution includes about 0.082% wt of the Poloxamer. In some embodiments, the Poloxamer is Poloxamer 188. In some embodiments, the cell suspension for cryopreservation and administration to a subject includes a cell cryopreservation and delivery solution that includes about 0.05% wt to about 0.5% wt recombinant human serum albumin. In some embodiments, the cell cryopreservation and delivery solution includes about 0.083% wt recombinant human serum albumin.

[00200] In some embodiments, the cell suspension for cryopreservation and administration to a subject includes a cell cryopreservation and delivery solution that includes

about 120 mM to about 190 mM dextrose; about 4 mM to about 40 mM HEPES; about 0.1 mM to about 2.5 mM calcium chloride; about 0.1 mM to about 1.5 mM magnesium chloride; about 4.0 mM to about 6.5 mM potassium chloride; about 20 mM to about 150 mM sodium chloride; about 0.1 mM to about 1.5 mM sodium phosphate monobasic; about 0.1% wt to about 20% wt Dextran; about 0.01% wt to about 20% wt Poloxamer; about 0.05% wt to about 0.5% recombinant human serum albumin; and about 5% wt to about 25% wt of the one or more cryoprotectants. In some embodiments, the one or more cryoprotectants consist essentially of DMSO. In some embodiments, the one or more cryoprotectants consists essentially of propylene glycol. In some embodiments, the Poloxamer is Poloxamer 188.

[00201] In some embodiments, the cell suspension for cryopreservation and administration to a subject includes a cell cryopreservation and delivery solution that includes about 24.6 mM dextrose; about 10.8 mM HEPES; about 1.8 mM calcium chloride; about 0.8 mM magnesium chloride; about 5.3 mM potassium chloride; about 83.1 mM sodium chloride; about 0.89 mM sodium phosphate monobasic; about 17.3% wt Dextran; about 0.082% wt Poloxamer; and about 10% wt of the one or more cryoprotectants. In some embodiments, the cell suspension for cryopreservation and administration to a subject includes a cell cryopreservation and delivery solution that includes about 22.1 mM dextrose; about 9.7 mM HEPES; about 1.6 mM calcium chloride; about 0.7 mM magnesium chloride; about 4.8 mM potassium chloride; about 75 mM sodium chloride; about 0.8 mM sodium phosphate monobasic; about 15.9% wt Dextran; about 0.07% wt Poloxamer; and about 10% wt of the one or more cryoprotectants. In some embodiments, the one or more cryoprotectants consists essentially of DMSO. In some embodiments, the one or more cryoprotectants consists essentially of propylene glycol. In some embodiments, the Poloxamer is Poloxamer 188.

[00202] In some embodiments, the cell suspension for cryopreservation and administration to a subject includes a cell cryopreservation and delivery solution that does not contain certain components, which are excluded wholly or below detectable limits. In some embodiments, the cell cryopreservation and delivery solution that does not include any components of animal origin. In some embodiments, the cell cryopreservation and delivery solution does not include one or more of zinc sulfate, and ferric nitrate. In some embodiments, the cell cryopreservation and delivery solution does not include sodium pyruvate. In some embodiments, the cell cryopreservation and delivery solution does not

include an amino acid. In some embodiments, the cell cryopreservation and delivery solution does not include a vitamin. In some embodiments, the cell suspension includes a pH indicator. In some embodiments, the pH indicator is Phenol Red.

[00203] In some embodiments, the cell suspension for cryopreservation and administration to a subject includes a cell cryopreservation and delivery solution that further includes one or more excipients. In some embodiments, the one or more excipients include one or more of polyethylene glycol, carboxymethyl cellulose, hyaluronic acid, starches, acrylates, methacrylates, polyvinyl alcohols, polyethylene oxides, polypropylene oxides, polyacrylates, polyvinylpyrrolidone, polymethacrylate, polylactic-co-glycolic acids, polyacrylamides, polylactides, chitosans, gums, guar gums, xanthan gums, carrageenans, hydroxypropyl cellulose, hydroxypropyl methyl cellulose, hydroxyethyl cellulose, cyclodextrin derivatives, beta-cyclodextrin derivatives, alginates, calcium alginates, dextran, pentasomaltose, hydroxyethyl starch, trehalose, sorbitol, mannitol, reduced glutathione, isoleucine, proline, ascorbic acid, N-acetyl-L-cysteine, carnitine, betaine, glycolipids, PEG lipids, lipoprotein, polysorbate 20, polysorbate 80, polyoxyl 15 hydroxy stearate, ectoine, and stearates. In some embodiments, the one or more excipients include polyvinylpyrrolidone. In some embodiments, the polyvinylpyrrolidone include one or more of polyvinylpyrrolidone K30, polyvinylpyrrolidone K12, polyvinylpyrrolidone K17, or polyvinylpyrrolidone K90. In some embodiments, the one or more excipients include hydroxyethyl starch. In some embodiments, the hydroxyethyl starch includes one or more of hydroxyethyl starch 130/0.4, hydroxyethyl starch 70/0.5, or hydroxyethyl starch 200/0.5. In some embodiments, the one or more excipients include polyethylene glycol. In some embodiments, the polyethylene glycol includes one or more of polyethylene glycol 300, polyethylene glycol 400, polyethylene glycol 600, polyethylene glycol 3350, or polyethylene glycol 4000. In some embodiments, the one or more excipients include dextran. In some embodiments, the dextran includes one or more of dextran 5, dextran 10, dextran 20, dextran 40, or dextran 70.

[00204] In some embodiments, the cell suspension for cryopreservation and administration to a subject includes a cell cryopreservation and delivery solution that further includes an additional cryoprotectant. In some embodiments, the additional cryoprotectant is a permeating-type cryoprotectant. In some embodiments, the permeating-type cryoprotectant includes dimethyl sulfoxide (DMSO), glycerol, or propylene glycol. In some embodiments, the only cryoprotectant present in the cell suspension is propylene glycol.

[00205] In some embodiments, the cell suspensions for cryopreservation and administration to a subject enables cryopreservation of a plurality of cells at a therapeutically effective dose. In some embodiments, the therapeutically effective dose includes about 10^0 cells to about 10^{10} cells. In some embodiments, the therapeutically effective dose includes a range of about 10^1 cells to about 10^9 cells, about 10^2 cells to about 10^8 cells, about 10^3 cells to about 10^7 cells, about 10^4 cells to about 10^6 cells. In some embodiments, the therapeutically effective dose includes about 10^4 to about 10^{10} cells, about 10^4 to about 10^5 cells, about 10^5 to about 10^9 cells, about 10^5 to about 10^6 cells, about 10^5 to about 10^7 cells, about 10^6 to about 10^7 cells, about 10^6 to about 10^8 cells, about 10^7 to about 10^8 , from about 10^8 to about 10^9 cells, about 10^8 to about 10^{10} cells, or about 10^9 to about 10^{10} cells. In some embodiments, the therapeutically effective dose includes a range of about 10^5 cells to about 10^8 cells. In some embodiments, the therapeutically effective dose includes a range of about 10^6 cells to about 10^8 cells. In some embodiments, the therapeutically effective dose includes at least 0.25 million cells, at least 0.5 million cells, at least 0.75 million cells, at least 1 million cells, at least 1.25 million cells, at least 1.5 million cells, at least 1.75 million cells, at least 2 million cells, at least 2.25 million cells, at least 2.5 million cells, at least 2.75 million cells, at least 3 million dopaminergic, at least 3.25 million cells, at least 3.5 million cells, at least 3.75 million cells, at least 4 million cells, at least 4.5 million cells, at least 5 million cells, at least 5.5 million cells, at least 6 million cells, at least 7 million cells, at least 8 million cells, at least 9 million cells, or at least 10 million or more cells. In some embodiments, the therapeutically effective dose includes at least 1.8 million cells. In some embodiments, the therapeutically effective dose includes at least 2.7 million cells. In some embodiments, the therapeutically effective dose includes at least 5.4 million cells. In some embodiments, the therapeutically effective dose includes at least 7 million cells.

[00206] In some embodiments, the cell suspensions for cryopreservation and administration to a subject enables stable storage of a plurality of cells. In some embodiments, the cell suspensions for cryopreservation and administration to a subject enables storage of a plurality of cells for up to at least about 1 hour to 24 hours, at least about 2 hours to 18 hours, at least about 4 hours to 12 hours, or at least about 6 hours to 10 hours at 4 °C. In some embodiments, the cell suspensions for cryopreservation and administration to a subject enables storage of a plurality of cells for up to at least about 2 hours, at least about 4 hours, at least about 6 hour, at least about 8 hours, at least about 10 hours, or at least about 12

hours at 4 °C. In some embodiments, the cell suspensions for cryopreservation and administration to a subject enables storage of a plurality of cells for up to at least about 6 hours at 4 °C. In some embodiments, the plurality of cells includes differentiated midbrain dopaminergic neuronal cells.

Methods of Preparing a Cell Suspension

[00207] Certain aspects of the disclosure provide methods of preparing cell suspensions for cryopreservation and administration to a subject (*e.g.*, a cell suspension for cryopreservation and administration to a subject).

[00208] In some embodiments, the method for preparing a cell suspension for cryopreservation and administration to a subject includes adding a cell cryopreservation and delivery solution (*e.g.*, a cell cryopreservation and delivery solution described herein) to a plurality of cells in a container, and mixing the plurality of cells and the cell cryopreservation and delivery solution to form a cell suspension (*e.g.*, a cell suspension for cryopreservation and administration to a subject described herein).

[00209] In some embodiments, the method for preparing a cell suspension for cryopreservation and administration to a subject includes adding a cell cryopreservation and delivery solution (*e.g.*, a cell cryopreservation and delivery solution described herein) to a plurality of cells that includes neural cells. In some embodiments, the neural cells include dopaminergic neurons. In some embodiments, the dopaminergic neurons include midbrain dopaminergic neurons, authentic midbrain dopaminergic neurons, midbrain dopaminergic neuron progenitor cells, dopaminergic neuron progenitor cells, or dopaminergic neuron precursor cells. In some embodiments, the plurality of cells includes differentiated midbrain dopaminergic neuronal cells, or a precursor thereof. In some embodiments, the differentiated midbrain dopaminergic neuronal cells are differentiated midbrain dopaminergic neuron progenitor cells.

[00210] In some embodiments, the method for preparing a cell suspension for cryopreservation and administration to a subject includes adding a cell cryopreservation and delivery solution (*e.g.*, a cell cryopreservation and delivery solution described herein) to a plurality of cells that is autologous to the subject. In other embodiments, the method for preparing a cell suspension for cryopreservation and administration to a subject includes

adding a cell cryopreservation and delivery solution (*e.g.*, a cell cryopreservation and delivery solution described herein) to a plurality of cells that is allogenic to the subject. In some embodiments, the subject is a human subject. In some embodiments, the plurality of cells includes human cells.

[00211] In some embodiments, the method for preparing a cell suspension for cryopreservation and administration to a subject includes adding a cell cryopreservation and delivery solution (*e.g.*, a cell cryopreservation and delivery solution described herein) to a plurality of cells in a container at a range of about 10^0 cells to about 10^{10} cells. In some embodiments, the plurality of cells in the container includes a range of about 10^1 cells to about 10^9 cells, about 10^2 cells to about 10^8 cells, about 10^3 cells to about 10^7 cells, about 10^4 cells to about 10^6 cells. In some embodiments, the plurality of cells includes about 10^4 to about 10^{10} cells, about 10^4 to about 10^5 cells, about 10^5 to about 10^9 cells, about 10^5 to about 10^6 cells, about 10^5 to about 10^7 cells, about 10^6 to about 10^7 cells, about 10^6 to about 10^8 cells, about 10^7 to about 10^8 , from about 10^8 to about 10^9 cells, about 10^8 to about 10^{10} cells, or about 10^9 to about 10^{10} cells. In some embodiments, the plurality of cells in the container includes a range of about 10^5 cells to about 10^8 cells. In some embodiments, the plurality of cells in the container includes a range of about 10^6 cells to about 10^8 cells. In some embodiments, the plurality of cells in the container includes at least 0.25 million cells, at least 0.5 million cells, at least 0.75 million cells, at least 1 million cells, at least 1.25 million cells, at least 1.5 million cells, at least 1.75 million cells, at least 2 million cells, at least 2.25 million cells, at least 2.5 million cells, at least 2.75 million cells, at least 3 million dopaminergic, at least 3.25 million cells, at least 3.5 million cells, at least 3.75 million cells, at least 4 million cells, at least 4.5 million cells, at least 5 million cells, at least 5.5 million cells, at least 6 million cells, at least 7 million cells, at least 8 million cells, at least 9 million cells, or at least 10 million or more cells. In some embodiments, the plurality of cells in the container includes at least 1.8 million cells. In some embodiments, the plurality of cells in the container includes at least 2.7 million cells. In some embodiments, the plurality of cells in the container includes at least 5.4 million cells. In some embodiments, the plurality of cells in the container includes at least 7 million cells.

[00212] In some embodiments, the volume of the cell suspension prepared by the method for preparing a cell suspension for cryopreservation and administration to a subject is about 0.1 mL to about 25 mL. In some embodiments, the volume of the cell suspension

prepared by the method for preparing a cell suspension for cryopreservation and administration to a subject is about 0.05 mL to about 20 mL. In some embodiments, the volume of the cell suspension prepared by the method for preparing a cell suspension for cryopreservation and administration to a subject is about 0.1 mL to about 0.2 mL, 0.2 mL to about 0.3 mL, 0.3 mL to about 0.4 mL, 0.4 mL to about 0.5 mL, 0.5 mL to about 0.6 mL, 0.6 mL to about 0.7 mL, 0.7 mL to about 0.8 mL, 0.9 mL to about 0.9 mL, or 0.9 mL to about 1 mL, 1 mL to 5 mL, 5 mL to 10 mL or 10 mL to 20 mL. In some embodiments, the volume of the cell suspension prepared by the method for preparing a cell suspension for cryopreservation and administration to a subject is about 0.1 mL. In some embodiments, the volume of the cell suspension prepared by the method for preparing a cell suspension for cryopreservation and administration to a subject is about 0.5 mL. In some embodiments, the volume of the cell suspension prepared by the method for preparing a cell suspension for cryopreservation and administration to a subject is about 1 mL.

[00213] In some embodiments, the plurality of cells has a low variability in dose sampling in the container. In some embodiments, the plurality of cells has a variability in dose sampling of less than about 5%, less than about 10%, less than about 15%, less than about 20%, less than about 25%, less than about 30%, less than about 35%, less than about 40%, less than about 45%, or less than about 50% in the container. In some embodiments, the plurality of cells has a variability in dose sampling of less than about 35% in the container. In some embodiments, the plurality of cells has a variability in dose sampling of less than about 25% in the container. In some embodiments, the plurality of cells has a variability in dose sampling of less than about 15% in the container.

[00214] In some embodiments, the method for preparing a cell suspension for cryopreservation and administration to a subject further includes cryopreserving the cell suspension. Cryopreservation can be performed by any suitable method known in the art. For instance, cryopreservation can include freezing cells or storing cells or refrigerating cells at above freezing temperatures. Cryopreservation can also include storing cells in a cell bank.

[00215] In some embodiments, the method for preparing a final cell suspension for cryopreservation and administration to a subject further includes thawing the cryopreserved cell suspension, and administering the thawed cell suspension to a subject. The cryopreserved cells can be thawed by any suitable method known in the art, such as, for

example, immersion in liquid at 37 °C. In some embodiments, the method does not include washing the cells with a cell wash buffer (*i.e.*, any solution that is used for washing the cells post thaw, for example, to remove the cryopreservation solution from cell suspension) after thawing the cells. In some embodiments, the cell wash buffer includes a phosphate buffer saline (PBS), Bio-Plex Pro™ Cell Signaling Wash Buffer, or Cultrex™ 3-D Cell Wash Buffer.

[00216] In some embodiments, this disclosure provides a method of preparing a cell cryopreservation and delivery solution. In some embodiments, the method comprises preparing a first solution comprising about 0.2% wt to about 20% wt dextran and about 20% wt to about 30% wt of one or more cryoprotectants; preparing a second solution; and combining substantially equal volumes of the first solution and the second solution to form the cell cryopreservation and delivery solution. In some embodiments, the second solution comprises about 145 mM to about 165 mM glucose. In some embodiments, the second solution comprises about 10 mM to about 15 mM HEPES. In some embodiments, the second solution comprises about 0.1 mM to about 2.5 mM calcium chloride. In some embodiments, the second solution comprises about 0.5 mM to about 1.0 mM magnesium chloride. In some embodiments, the second solution comprises about 4.0 mM to about 6.0 mM potassium chloride. In some embodiments, the second solution comprises about 60 mM to about 80 mM sodium chloride.

[00217] In some embodiments, this disclosure provides a method of preparing a cell suspension for cryopreservation and delivery to a subject. In some embodiments, the method comprises suspending a plurality of cells in an isotonic solution to form a cell suspension; preparing a concentrated cryoprotectant solution comprising about 20% wt to about 30% wt of one or more cryoprotectants; and gradually adding the concentrated cryoprotectant solution to the cell suspension to form the final cell suspension. In some embodiments, the isotonic solution comprises about 145 mM to about 165 mM glucose. In some embodiments, the isotonic solution comprises about 10 mM to about 15 mM HEPES. In some embodiments, the isotonic solution comprises about 0.1 mM to about 2.5 mM calcium chloride. In some embodiments, the isotonic solution comprises about 0.5 mM to about 1.0 mM magnesium chloride. In some embodiments, the isotonic solution comprises about 4.0 mM to about 6.0 mM potassium chloride. In some embodiments, the isotonic solution comprises about 60 mM to about 80 mM sodium chloride. In some embodiments, the

concentrated cryoprotectant solution further comprises about 0.2% wt to about 20% wt dextran.

IV. Methods of Treatment

[00218] Certain aspects of the disclosure provide methods for treating a subject. In some embodiments, provided herein is a method for treating a subject includes thawing a cell suspension (*e.g.*, a cell suspension described herein), and administering a dose of the cell suspension to the subject. In some embodiments, the method for treating a subject does not include a wash step after thawing. In some embodiments, the wash step includes washing with a phosphate buffer saline (PBS), Bio-Plex Pro™ Cell Signaling Wash Buffer, or Cultrex™ 3-D Cell Wash Buffer.

[00219] In some embodiments, the method for treating a subject further includes diluting the cell suspension after thawing. In some embodiments, the cell suspension is diluted with a cell delivery solution not including a cryoprotectant after thawing.

[00220] In some embodiments, the method for treating a subject does not include diluting the cell suspension after thawing.

[00221] In some embodiments, the method for treating a subject further comprises maintaining the cell suspension (*e.g.*, at 4 °C storage or on ice) at for a period of time after thawing the cells. In some embodiments, the cell suspension is maintained for up to at least about 6 hours at 4 °C after thawing and prior to administration to a subject.

[00222] In some embodiments, the method for treating a subject further includes the step of administering one or more additional doses of the cell suspension. For instance, the method can include administering one, two, three, four or more additional doses of the cell suspension over the course of treatment of the subject. The one or more additional doses can be administered using the same or a different route of administration to the initial dose of the cell suspension, and can contain the same amount or a different amount of the cell suspension.

[00223] In some embodiments, the cell suspension used in the method for treating a subject includes a plurality of cells that includes neural cells. In some embodiments, the

neural cells include dopaminergic neurons. In some embodiments, the dopaminergic neurons include midbrain dopaminergic neurons, authentic midbrain dopaminergic neurons, midbrain dopaminergic neuron progenitor cells, dopaminergic neuron progenitor cells, or dopaminergic neuron precursor cells. In some embodiments, the plurality of cells includes differentiated midbrain dopaminergic neuronal cells, or a precursor thereof. In some embodiments, the differentiated midbrain dopaminergic neuronal cells are differentiated midbrain dopaminergic neuron progenitor cells.

[00224] In some embodiments, the cell suspension used in the method for treating a subject includes a plurality of cells that is derived from stem cells. In some embodiments, the stem cells are pluripotent stem cells. In some embodiments, the pluripotent stem cells are embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs), or a combination thereof. In some embodiments, the stem cells are multipotent stem cells. In some embodiments, the stem cells are human stem cells.

[00225] In some embodiments, the cell suspension used in the method for treating a subject includes a plurality of cells at a range of about 10^0 cells to about 10^{10} cells. In some embodiments, the plurality of cells includes a range of about 10^1 cells to about 10^9 cells, about 10^2 cells to about 10^8 cells, about 10^3 cells to about 10^7 cells, about 10^4 cells to about 10^6 cells. In some embodiments, the plurality of cells includes about 10^4 to about 10^{10} cells, about 10^4 to about 10^5 cells, about 10^5 to about 10^9 cells, about 10^5 to about 10^6 cells, about 10^5 to about 10^7 cells, about 10^6 to about 10^7 cells, about 10^6 to about 10^8 cells, about 10^7 to about 10^8 , from about 10^8 to about 10^9 cells, about 10^8 to about 10^{10} cells, or about 10^9 to about 10^{10} cells. In some embodiments, the plurality of cells includes a range of about 10^5 cells to about 10^8 cells. In some embodiments, the plurality of cells includes at least 0.25 million cells, at least 0.5 million cells, at least 0.75 million cells, at least 1 million cells, at least 1.25 million cells, at least 1.5 million cells, at least 1.75 million cells, at least 2 million cells, at least 2.25 million cells, at least 2.5 million cells, at least 2.75 million cells, at least 3 million dopaminergic, at least 3.25 million cells, at least 3.5 million cells, at least 3.75 million cells, at least 4 million cells, at least 4.5 million cells, at least 5 million cells, at least 5.5 million cells, at least 6 million cells, at least 7 million cells, at least 8 million cells, at least 9 million cells, or at least 10 million or more cells.

[00226] In some embodiments, the cell suspension used in the method for treating a subject includes a plurality of cells that is autologous to the subject. In other embodiments, the cell suspension used in the method for treating a subject includes a plurality of cells that is allogenic to the subject.

[00227] In some embodiments, the cell suspension used with the method for treating a subject includes one or more of: one or more energy source components, one or more salts, and one or more cryoprotectants.

[00228] In some embodiments, the cell suspension used with the method for treating a subject includes about 0.5 mM to about 280 mM of the one or more energy source components. In some embodiments, the cell suspension includes about 25 mM to about 280 mM of the one or more energy source components. In some embodiments, the cell suspension includes about 20 mM to about 30 mM of the one or more energy source components. In some embodiments, the cell suspension includes about 24.6 mM of the one or more energy source. In some embodiments, the cell suspension includes about 22.1 mM of the one or more energy source. In some embodiments, the one or more energy source components include dextrose or glucose.

[00229] In some embodiments, the cell suspension for cryopreservation and administration to a subject includes a cell cryopreservation and delivery solution that includes about 0.1 mM to about 200 mM of each of the one or more salts. In some embodiments, the cell suspension for cryopreservation and administration to a subject includes a cell cryopreservation and delivery solution that includes about 10 mM to about 200 mM of each of the one or more salts. In some embodiments, the one or more salts includes one or more of calcium chloride, magnesium chloride, potassium chloride, sodium chloride and sodium phosphate or a combination thereof. In some embodiments, the cell suspension for cryopreservation and administration to a subject includes a cell cryopreservation and delivery solution that includes about 0.1 mM to about 2.5 mM of calcium chloride. In some embodiments, the cell suspension for cryopreservation and administration to a subject includes a cell cryopreservation and delivery solution that includes about 0.1 mM to about 1.5 mM of magnesium chloride. In some embodiments, the cell cryopreservation and delivery solution includes about 0.5 mM to 1.0 mM of magnesium chloride. In some embodiments, the cell suspension for cryopreservation and administration to a subject includes a cell cryopreservation and delivery solution that includes about 4.0 mM to about 6.5 mM

potassium chloride. In some embodiments, the cell cryopreservation and delivery solution includes about 4.0 mM to 6 mM potassium chloride. In some embodiments, the cell suspension for cryopreservation and administration to a subject includes a cell cryopreservation and delivery solution that includes about 20 mM to about 150 mM sodium chloride. In some embodiments, the cell cryopreservation and delivery solution includes about 60 mM to 80 mM sodium chloride. In some embodiments, the cell suspension used with the method for treating a subject includes about 0.1 mM to about 200 mM of each of the one or more salts. In some embodiments, the one or more salts include one or more of calcium chloride, magnesium chloride, potassium chloride, sodium chloride and sodium phosphate monobasic, or a combination thereof. In some embodiments, the cell suspension includes a cell suspension that includes about 1.0 mM to about 2.5 mM of calcium chloride. In some embodiments, cell suspension includes about 1.6 mM of calcium chloride. In some embodiments, cell suspension includes about 1.8 mM of calcium chloride. In some embodiments, the cell suspension for cryopreservation and administration to a subject includes a cell suspension that includes about 0.1 mM to about 1.5 mM of magnesium chloride. In some embodiments, the cell suspension includes about 0.7 mM of magnesium chloride. In some embodiments, the cell suspension includes about 0.8 mM of magnesium chloride. In some embodiments, the cell suspension for cryopreservation and administration to a subject includes a cell suspension that includes about 4.5 mM to about 6.5 mM potassium chloride. In some embodiments, the cell suspension includes about 4.8 mM potassium chloride. In some embodiments, the cell suspension includes about 5.3 mM potassium chloride. In some embodiments, the cell suspension for cryopreservation and administration to a subject includes a cell suspension that includes about 40 mM to about 150 mM sodium chloride. In some embodiments, the cell suspension includes about 75 mM sodium chloride. In some embodiments, the cell suspension includes about 83.1 mM sodium chloride. In some embodiments, the cell suspension for cryopreservation and administration to a subject includes a cell suspension that includes about 0.1 mM to about 1.5 mM sodium phosphate monobasic. In some embodiments, the cell suspension includes about 0.8 mM sodium phosphate monobasic. In some embodiments, the cell suspension includes about 0.89 mM sodium phosphate monobasic.

[00230] In some embodiments, the cell suspension used with the method for treating a subject includes about 5% weight by volume (wt) to about 20% wt of the one or more cryoprotectants. In some embodiments, the cell suspension used with the method for

treating a subject includes about 1% weight by volume (wt) to about 30% wt of the one or more cryoprotectants. In some embodiments, the cell suspension includes about 10% wt of the one or more cryoprotectants. In some embodiments, the cell suspension includes about 15% wt of the one or more cryoprotectants. In some embodiments, the one or more cryoprotectants consists essentially of DMSO. In some embodiments, the one or more cryoprotectants consists essentially of propylene glycol.

[00231] In some embodiments, the cell suspension used with the method for treating a subject further includes one or more pH buffers. In some embodiments, the cell suspension for cryopreservation and administration to a subject includes a cell suspension that includes about 5 mM to about 50 mM of the one or more pH buffers. In some embodiments, the cell suspension includes about 10 mM to 15 of the one or more pH buffers. In some embodiments, the cell suspension includes about 10.8 mM of the one or more pH buffers. In some embodiments, the one or more pH buffers comprise 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 3-(N-morpholino)propanesulfonic acid (MOPS), 2-(N-morpholino) ethanesulfonic acid (MES), bicarbonate, or phosphates, or a combination as pH buffers.

[00232] In some embodiments, the cell suspension used with the method for treating a subject (*e.g.*, a method for treating a subject provided herein) further includes one or more stabilizing agents. In some embodiments, the cell suspension for cryopreservation and administration to a subject includes a cell suspension that includes about 0.01% wt to about 25% wt of the one or more stabilizing agents. In some embodiments, the one or more stabilizing agents include a non-permeable type cryoprotectant. In some embodiments, the one or more stabilizing agents includes dextran, poloxamer, one or more antioxidants, or a recombinant human serum albumin. In some embodiments, the cell suspension for cryopreservation and administration to a subject includes a cell suspension that includes about 5% wt to about 20% wt of Dextran. In some embodiments, the cell suspension includes about 17.3% wt Dextran. In some embodiments, the cell suspension for cryopreservation and administration to a subject includes a cell suspension that includes about 0.01% wt to about 20% wt of the Poloxamer. In some embodiments, the cell suspension includes about 0.07% wt of the Poloxamer. In some embodiments, the cell suspension includes about 0.082% wt of the Poloxamer. In some embodiments, the Poloxamer is Poloxamer 188. In some embodiments, the cell suspension for cryopreservation

and administration to a subject includes a cell suspension that includes about 0.05% wt to about 0.5% wt recombinant human serum albumin. In some embodiments, the cell suspension includes about 0.083% wt recombinant human serum albumin.

[00233] In some embodiments, In some embodiments, the cell suspension used with the method for treating a subject includes about 145 mM to about 165 mM glucose, about 10 mM to about 15 mM HEPES, about 0.1 mM to about 2.5 mM calcium chloride, about 0.5 mM to about 1.0 mM magnesium chloride, about 4.0 mM to about 6.0 mM potassium chloride, about 60 mM to about 80 mM sodium chloride, 20 mM to about 30 mM dextrose; about 10 mM to about 50 mM HEPES; about 1.0 mM to about 2.5 mM calcium chloride; about 0.1 mM to about 1.5 mM magnesium chloride; about 4.5 mM to about 6.5 mM potassium chloride; about 40 mM to about 150 mM sodium chloride; about 0.1 mM to about 1.5 mM sodium phosphate monobasic; about 0.1% wt to about 10% wt Dextran; about 0.01% wt to about 20% wt Poloxamer; about 0.05% wt to about 0.5% recombinant human serum albumin; and about 5% wt to about 25% wt of the one or more cryoprotectants. In some embodiments, the one or more cryoprotectants consist essentially of DMSO. In some embodiments, the one or more cryoprotectants consists essentially of propylene glycol.

[00234] In some embodiments, the cell suspension used with the method for treating a subject includes about 24.6 mM dextrose; about 10.8 mM HEPES; about 1.8 mM calcium chloride; about 0.8 mM magnesium chloride; about 5.3 mM potassium chloride; about 83.1 mM sodium chloride; about 0.89 mM sodium phosphate monobasic; about 17.3% wt Dextran; about 0.082% wt Poloxamer; about 0.083% wt recombinant human serum albumin; and about 10% wt of the one or more cryoprotectants. In some embodiments, the cell suspension includes about 22.1 mM dextrose; about 9.7 mM HEPES; about 1.6 mM calcium chloride; about 0.7 mM magnesium chloride; about 4.8 mM potassium chloride; about 75 mM sodium chloride; about 0.8 mM sodium phosphate monobasic; about 15.9% wt Dextran; about 0.07% wt Poloxamer; and about 10% wt of the one or more cryoprotectants. In some embodiments, the one or more cryoprotectants consists essentially of DMSO. In some embodiments, the one or more cryoprotectants consists essentially of propylene glycol. In some embodiments, the Poloxamer is Poloxamer 188.

[00235] In some embodiments, the cell suspension used with the method for treating a subject does not include certain components, which are excluded wholly or below detectable limits. In some embodiments, the cell suspension that does not include any

components of animal origin. In some embodiments, the cell suspension does not include one or more of zinc sulfate, sodium bicarbonate, and ferric nitrate. In some embodiments, the cell suspension does not include sodium pyruvate. In some embodiments, the cell suspension does not include an amino acid. In some embodiments, the cell suspension does not include a vitamin.

[00236] In some embodiments, the cell suspension used with the method for treating a subject includes a pH indicator. In some embodiments, the pH indicator is Phenol Red.

[00237] In some embodiments, the cell suspension used with the method for treating a subject further includes one or more excipients. In some embodiments, the one or more excipients include one or more of polyethylene glycol, carboxymethyl cellulose, hyaluronic acid, starches, acrylates, methacrylates, polyvinyl alcohols, polyethylene oxides, polypropylene oxides, polyacrylates, polyvinylpyrrolidone, polymethacrylate, polylactic-co-glycolic acids, polyacrylamides, polylactides, chitosans, gums, guar gums, xanthan gums, carrageenans, hydroxypropyl cellulose, hydroxypropyl methyl cellulose, hydroxyethyl cellulose, cyclodextrin derivatives, beta-cyclodextrin derivatives, alginates, calcium alginates, dextran, pentaisomaltose, hydroxyethyl starch, trehalose, sorbitol, mannitol, reduced glutathione, isoleucine, proline, ascorbic acid, N-acetyl-L-cysteine, carnitine, betaine, glycolipids, PEG lipids, lipoprotein, polysorbate 20, polysorbate 80, polyoxyl 15 hydroxy stearate, ectoine, and stearates. In some embodiments, the one or more excipients include polyvinylpyrrolidone. In some embodiments, the polyvinylpyrrolidone include one or more of polyvinylpyrrolidone K30, polyvinylpyrrolidone K12, polyvinylpyrrolidone K17, or polyvinylpyrrolidone K90. In some embodiments, the one or more excipients include hydroxyethyl starch. In some embodiments, the hydroxyethyl starch includes one or more of hydroxyethyl starch 130/0.4, hydroxyethyl starch 70/0.5, or hydroxyethyl starch 200/0.5. In some embodiments, the one or more excipients include polyethylene glycol. In some embodiments, the polyethylene glycol includes one or more of polyethylene glycol 300, polyethylene glycol 400, polyethylene glycol 600, polyethylene glycol 3350, or polyethylene glycol 4000. In some embodiments, the one or more excipients include dextran. In some embodiments, the dextran includes one or more of dextran 5, dextran 10, dextran 20, dextran 40, or dextran 70.

[00238] In some embodiments, the cell suspension used with the method for treating a subject further includes an additional cryoprotectant. In some embodiments, the additional cryoprotectant is a permeating-type cryoprotectant. In some embodiments, the permeating-type cryoprotectant includes dimethyl sulfoxide (DMSO), glycerol, or propylene glycol.

[00239] The administration of the cell suspension can be performed by any suitable method known in the art. The cell suspension of the methods described herein can be administered to a subject by any suitable route of administration. Methods of administration of populations of engineered cells described herein are known to those in the art. In some embodiments, the method includes administering the cell suspension orally, pulmonarily, intranasally, parenterally (intravenously, intramuscularly, intraperitoneally, or subcutaneously), rectally, intralymphatically, or topically. In some embodiments, the cell suspension is administered injection. In some embodiments, the cell suspension is administered by direct injection into a portion of the subject.

[00240] In some embodiments, the administering step includes loading a dose delivery device with the dose of the cell suspension, and injecting the dose to the subject. In some embodiments, the injecting occurs directly into a portion of the brain of the subject. In some embodiments, the portion of the brain includes a putamen. In other embodiments, the injecting step occurs into an intravenous line. In some embodiments, the intravenous line is in a blood vessel of the subject. The dose delivery device may be any suitable dose delivery device known in the art. In some embodiments, the dose delivery device is a syringe.

[00241] In some embodiments, the method for treating a subject further includes a step of administering an additional treatment. In some embodiments, the step of administering an additional treatment is concurrent to the administration of the dose of the cell suspension. In some embodiments, the step of administering an additional treatment is subsequent to the administration of the dose of the cell suspension. In some embodiments, the additional treatment includes a chemotherapeutic agent, a radiation treatment, a bone marrow treatment, a hormone treatment, or a surgical treatment.

[00242] In some embodiments, the subject is a human.

[00243] In some embodiments, the method for treating a subject is for treating Parkinson's disease in the subject. In some embodiments, the Parkinson's disease is early

Parkinson's disease. In some embodiments, the Parkinson's disease is advanced Parkinson's disease.

EXAMPLES

[00244] In order that this disclosure may be better understood, the following examples are set forth. These examples are for purposes of illustration only and are not to be construed as limiting the scope of the disclosure in any manner.

Example 1. Screening cryopreservation and delivery solutions

[00245] This example describes experiments conducted to assess the efficacy of cryopreservation and delivery solutions for preserving therapeutic cells for subsequent use. One objective was to determine the efficacy of various cryopreservation solutions in preserving cell viability and functionality post-cryopreservation while eliminating the need for post-thaw washing steps. To achieve this, a protocol involving the thawing and resuspension of cryopreserved cells, followed by subsequent treatment and evaluation was conducted. The protocol was designed to exemplify a "thaw-to-inject" cell therapy approach by not requiring any post-thaw wash step, which can be used for streamlining methods for administering therapeutic cells to patients.

[00246] Cryopreserved neural cells were thawed and resuspended by pipetting. The resuspended neural cells were aliquoted into multiple sample tubes and combined with one of twenty-six different test cryopreservation solutions (**Table 1**), or a cryopreservation solution of CDS + Propylene Glycol, STEM-CELLBANKER, or STEM-CELLBANKER DMSO Free (**Table 2**). The combined cell solutions (approximately 50µL in volume each) were transferred to cryovials and cryopreserved using controlled rate freezing. The frozen cell vials were then transferred to LN₂ vapour-phase storage for a minimum of 48 hours.

Table 1. Test cryopreservation and delivery solutions.

Sample identification	Compositions (mg component/g formulation)																													
	aCSF	Ascorbic acid	Calcium chloride dihydrate	Cryosior CSB	Dextran 40	DMSO	DPBS (-/-)	DPBS (+/+)	Wash solution	Wash solution - rHSA	Delivery Solution - rHSA	Glucose monohydrate	HA 1.6 MPa	HBSS (-/-)	HEPES	HT-FRS	Human serum albumin 25% solution	Isolucine	Magnesium chloride hexahydrate	Nutristor	P188	PentaHibc 40% solution	Potassium chloride	Propylene glycol	PVA 80% 9000-10000 Da	rHSA	Sodium chloride	Sodium dithyrogen phosphate dihydrate	Water	
F1	0	0	0.26	0	50	0	0	0	0	0	0	34.8	20	0	2.54	0	0	0.98	0.16	0	0	0	0	0.39	100	1	0.99	7.31	0.14	781
F2	0	0	0.26	0	50	0	0	0	0	0	0	33	2	0	2.54	0	0	0.98	0.16	0	10	0	0	0.39	100	0	0.99	0.05	0.14	800
F3	0	0	0.26	0	50	0	0	0	0	0	0	33	0	0	2.54	0	0	0.98	0.16	0	10	0	0	0.39	100	0	0.99	0.05	0.14	802
F4	0	0	0	0	0	20	0	0	0	0	0	0	0	0	0	0	160	0	0	0	0	0	250	0	0	0	0	0	0	570
F5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	160	0	0	0	0	0	250	0	20	0	0	0	0	570
F6	0	0	0	900	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0	0
F7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	900	0	0	0	0	0	0	0	100	0	0	0	0	0	0
F8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	900	0	0	0	100	0	0	0	0	0	0
F9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0	900
F10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	0	0	0	5	0	895
F11	0	0	0	0	0	0	0	0	0	0	0	55	0	0	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0	845
F12	0	0	0	0	0	0	0	0	0	0	0	0	0	900	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0	0
F13	0	0	0	0	0	0	900	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0	0
F14	0	0	0	0	0	0	0	900	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0	0
F15	900	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0	0
F16	0	0	0	0	0	0	0	0	900	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0	0
F17	0	0	0	0	0	0	0	0	0	900	0	0	0	0	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0	0
F18	0	0	0	0	156	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	1	0	0	100	0	0	0	0	0	739
F19	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	1	0	0	100	0	0	0	0	0	895
F20	0	0	0	0	156	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	100	0	0	0	0	0	743
F21	0	0	0	0	156	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0	740
F22	0	0	0	0	156	0	739	0	0	0	0	4	0	0	0	0	0	0	0	0	1	0	0	100	0	0	0	0	0	0
F23	0	0	0	0	0	0	895	0	0	0	0	4	0	0	0	0	0	0	0	0	1	0	0	100	0	0	0	0	0	0

Table 2. Cryopreservation and delivery solutions: CDS Propylene Glycol & STEM-CELLBANKER

Component	Function	Candidate Formulations			
		STEM-CELLBANKER	STEM-CELLBANKER DMSO Free	CDS + 10% DMSO	CDS + 10% Propylene Glycol
Dimethyl sulfoxide (DMSO)	Cryoprotectant	10%	0%	10%	0%
Propylene glycol	Cryoprotectant	ND	10%	0%	10%
d-Glucose (Dextrose)	Source of energy	ND	ND	0.41%	0.41%
Poloxamer 188	Stabilizing agent	ND	ND	0.07%	0.07%
Dextran 40	Stabilizing agent	ND	ND	15.56%	15.56%
HEPES	pH buffering agent	ND	ND	0.21%	0.21%
Calcium chloride	Salt (osmotic balance)	ND	ND	0.02%	0.02%
Magnesium chloride	Salt (osmotic balance)	ND	ND	0.01%	0.01%
Potassium chloride	Salt (osmotic balance)	ND	ND	0.03%	0.03%
Sodium chloride	Salt (osmotic balance)	ND	ND	0.40%	0.40%
Sodium phosphate monobasic	Salt (osmotic balance)	ND	ND	0.01%	0.01%

[00247] Prior to evaluation, the frozen cell vials were retrieved from LN₂ and placed on dry ice for transport. The frozen vials were briefly thawed in a 37°C water bath and stored at 2–8°C for approximately 6 hours. The cell vials were transferred to a biosafety cabinet (BSC) and pre-warmed media (~950µL) was slowly added to the cryovials. The cells were evenly suspended by pipette, and samples were withdrawn and used to measure live cell concentration and viability (**FIGS. 1A-B**) on a NucleoCounter NC-200. Measurement of live cell concentration provides a comparison of the number of viable neural cells present after cryopreservation and thawing of cells in the different solutions, while viability indicates the proportion of live cells compared to the total cell count, reflecting the solutions' ability to maintain cell health.

[00248] After measuring live cell concentrations and viability, the remaining cells were moved into the BSC and plated onto cell culture dishes. The culture dishes were covered with aluminum foil and briefly incubated at room temperature, after which the culture dishes were returned to the incubator.

[00249] After 18–24 hours of incubation, the culture dishes were transferred to a BSC. The media was removed and replaced with staining media: culture medium containing NucLight Rapid Red stain (diluted 500x) and 1 µM of elacridar. The plate was placed in an Incucyte, and a scan was performed 1 hour after the addition of the staining media. The scan collected phase and red fluorescence images, which were used to count the number of adhered live cells per unit of area. This plated density was divided by the density of plated live cells to yield the replating efficiency (**FIG. 1C**). Replating efficiency assesses the ability of the solutions to support the adhesion and growth of thawed cells, providing valuable information on their functional recovery.

Example 2: Development and evaluation of cryopreservation and delivery solutions

[00250] This example describes experiments conducted to evaluate the effectiveness of cryopreservation and delivery solutions for storing and using neural cells.

[00251] Stem cells were differentiated into neural cells. The neural cells were divided into five centrifuge tubes and pelleted. The supernatant was removed, and the cells were resuspended in different cryopreservation and delivery solutions. After resuspension, the cells were transferred into pre-chilled cryovials (1 mL per vial for the control cell suspension for injection or “CSI” group, and 0.1 mL per vial for the test groups). The vials were

transferred into a pre-chilled CRF instrument and frozen. The cryopreserved cells were then transferred to LN₂ storage for a minimum of 48 hours.

[00252] Prior to evaluation, the frozen cell vials were retrieved from LN₂ and placed on dry ice for transport. For the control samples, *i.e.*, the “CSI” vials, a wash step was performed to remove cryopreservation materials. Then, the control cells were resuspended in cell delivery solution (CDS), and the concentration was adjusted to a final concentration of approximately 100 million cells/mL.

[00253] The cell vials from the test groups (*i.e.*, STEM-CELLBANKER (SCB), SCB DMSO free, CDS + 10% DMSO, and CDS + 10% PG) were transferred to a biosafety cabinet (BSC), and two 45 µL aliquots were taken (for 0hr and 6hr testing), which were then diluted with approximately 405 µL of pre-warmed Neurobasal media, which was slowly added. The dilution was performed so that the cells could be counted. The cells were evenly suspended by pipette, and three 100 µL samples were withdrawn and used to measure cell concentration and viability on a NucleoCounter NC-200 (**FIGS. 2A-B**). Another 100 µL of the cell suspension was transferred into test tubes, and the cells were centrifuged to pellet the cells. The supernatant was carefully removed, and the cells were resuspended in 1 mL of culture media. Dilutions of this stock suspension were created (3x, 6x, or 30x) by diluting the stock with additional culture media. Next, 100 µL of each of the four concentrations (stock and three dilutions) of cell suspensions were added to culture dishes. The culture dishes were covered with aluminum foil and left at room temperature for 15 min, after which they were returned to the incubator. This testing was repeated approximately 6 h after thaw with the remaining cell aliquots.

[00254] After 18–24 h of incubation, the plates were transferred to a BSC. The media was removed and replaced with staining media: culture medium containing NucLight Rapid Red stain (diluted 500x) and 1 µM of elacridar. The plates were placed in an Incucyte, and a scan was scheduled for 1 h after the addition of the staining media. The scan collected phase and red fluorescence images, which were used to count the number of adhered live cells per unit of area. Linear regressions were performed on the linear section of a scatter plot of actual vs. expected plated density, from which replating efficiencies were extracted as 100% times the slopes (**FIG. 2C**)

Example 3: Evaluation of Various Polymers and Concentrations Thereof for Cell Cryopreservation and Delivery Solutions

[00255] This example describes experimental work that was conducted to evaluate different polymer types and concentrations for cell cryopreservation and delivery solutions.

[00256] In initial studies, different polymer types were compared to identify optimal candidates for the cryopreservation and delivery solution. Neural cells (*i.e.*, dopaminergic neuronal precursor cells) were combined with test cryopreservation and delivery solutions containing different polymers. Different molecular weight polyvinylpyrrolidones (PVP K12, PVP K30) and dextran (Dextran 40 and Dextran 70) were compared for their post-thaw viability and replating efficiency (**FIGS. 3A-3B**), as described in Example 1. Under the conditions tested, Dextran type polymers showed a modest improvement in terms of replating efficiency as compared to polyvinylpyrrolidones.

[00257] Studies were then conducted to assess the impact of varying polymer concentrations. Various concentrations of Dextran 40 (0%, 1%, 5%, 10%, and 15%) were evaluated for post-thaw cell viability and replating efficiency (**FIGS. 4A-4B**). The data demonstrated reduced cell viability and replating efficiency at Dextran 40 concentrations greater than 10%.

[00258] Additional studies evaluated different molecular weights of polyvinylpyrrolidone (PVP) (**FIGS. 5A-5D**) and compared Dextran 70 as an alternative to Dextran 40 (**FIGS. 6A-6B**). Viability and functionality assessments were performed, *i.e.*, by evaluating post-thaw viability and determining replating efficiency. Prior to evaluation, combined cell solutions were transferred to cryovials and cryopreserved using controlled rate freezing. The frozen cell vials were then transferred to LN2 vapour-phase storage. Post-thaw cell viability was assessed at 0 and 6 hours after thawing using a NucleoCounter NC-200. Replating efficiency was evaluated by plating the thawed cells onto culture dishes and analyzing cell adherence after 18-24 hours using NucLight Rapid Red staining.

Example 4: Evaluation of Glucose Concentration in Cell Cryopreservation and Delivery Solutions

[00259] This example describes experiments conducted to evaluate the impact of glucose concentration in cell cryopreservation and delivery solutions.

[00260] Neural cells (i.e., dopaminergic neuronal precursor cells) were combined with test cell cryopreservation and delivery solutions containing different glucose concentrations (3% versus 0.5%).

[00261] FIGS. 7A-7B show exemplary experimental results demonstrating glucose concentration impacted post-thaw cell viability and replating efficiency. Both formulations showed improved performance with 3% glucose as compared to 0.5% glucose.

[00262] These findings were further validated in studies comparing numbered exemplary cryopreservation and delivery solutions (i.e., Formulation #47, #49, #50, and #51), which varied in glucose concentration (FIGS. 8A-8B). The results demonstrated improved performance of formulations containing 3% glucose.

Example 5: Optimization of Propylene Glycol Concentration in Cell Cryopreservation and Delivery Solutions

[00263] This example describes experiments conducted to evaluate propylene glycol (PG) concentration in cell cryopreservation and delivery solutions.

[00264] Neural cells (i.e., dopaminergic neuronal precursor cells) were combined with test solutions containing different PG concentrations in a base formulation of about 3% glucose. Initial studies compared 8% versus 10% PG with 1% Dextran 40. The combined cell solutions were transferred to cryovials and cryopreserved using controlled rate freezing. The frozen cell vials were then transferred to LN2 vapour-phase storage.

[00265] Post-thaw cell viability was assessed at 0 and 6 hours after thawing using a NucleoCounter NC-200 (FIG. 9A). Replating efficiency was evaluated by plating the thawed cells onto culture dishes and analyzing cell adherence after 18-24 hours using NuLight Rapid Red staining (FIG. 9B).

[00266] Further studies evaluated the impact of higher PG concentrations (10% versus 12.5%) in formulations containing 1% Dextran 40 (FIGS. 10A-10B). The data demonstrated improved replating efficiency with 12.5% PG concentration as compared to 10% PG, while maintaining comparable cell viability.

[00267] The effect of PG concentration was also assessed with varying Dextran 40 concentrations. As shown in FIGS. 11A-11B, cell viability and replating efficiency were

compared between formulations containing either 10% or 12.5% PG combined with 1% or 5% Dextran 40. The results indicated that higher PG concentration (12.5%) improved preservation outcomes across different Dextran 40 concentrations.

[00268] Additional validation studies (**FIGS. 12A-12B**) demonstrated improved post-thaw cell viability and replating efficiency with 12.5% PG as compared to 10% PG in formulations containing 1% Dextran 40.

[00269] Additional studies were conducted to compare different formulations and cryoprotectants. **FIGS. 13A-13B** show comparative data between four formulations: Formulation #47 (10% propylene glycol, 1% Dextran 40, 3% glucose), Formulation #49 (10% propylene glycol, 1% Dextran 40, 0.5% glucose), Formulation #50 (12.5% propylene glycol, 5% Dextran 40, 3% glucose), and Formulation #51 (12.5% propylene glycol, 5% Dextran 40, 0.5% glucose). Post-thaw cell viability (**FIG. 13A**) and replating efficiency (**FIG. 13B**) were assessed, with no significant differences observed between formulations.

[00270] The performance of different penetrating cryoprotectants was also evaluated. **FIGS. 14A-14C** compare formulations containing either 10% propylene glycol or 10% glycerol. Cell viability (**FIG. 14A**) and replating efficiency (**FIG. 14B**) were assessed, while **FIG. 14C** shows the controlled rate freezing temperature profiles over a 60-minute freezing process, comparing the chamber temperature with the temperature profiles of solutions containing either propylene glycol or glycerol.

[00271] Further studies assessed the stability of different cryoprotective agents. **FIGS. 15A-15B** compare formulations containing either 10% propylene glycol or 10% glycerol with 5% Dextran 40. Post-thaw cell viability was measured pre-cryopreservation, immediately after thawing (0 hour), and after 6 hours (**FIG. 15A**). Replating efficiency was evaluated at both 0 and 6 hours post-thaw (**FIG. 15B**).

Example 6: Effect of Salt Concentration and Osmolality on Cell Cryopreservation and Delivery Solutions

[00272] This example describes experiments conducted to evaluate the impact of NaCl concentration and resulting osmolality for cell cryopreservation and delivery solutions.

[00273] Neural cells (*i.e.*, dopaminergic neuronal precursor cells) were combined with test solutions containing different NaCl concentrations. The combined cell solutions were transferred to cryovials and cryopreserved using controlled rate freezing. The frozen cell vials were then transferred to LN2 vapour-phase storage.

[00274] Initial studies evaluated various NaCl concentrations (0.72%, 0.55%, 0.36%, and 0.06%) to assess the impact on cell viability and functionality. Post-thaw cell viability and replating efficiency were measured (**FIGS. 16A-16D; and FIGS. 18A-18B**). The data demonstrated that NaCl concentration impacted both cell viability and replating outcomes.

[00275] Further studies compared formulations with different osmolalities (~1900 mOsm/kg versus ~2100 mOsm/kg), achieved by varying NaCl concentration (0.36% versus 0.72%). Cell viability was assessed pre-cryopreservation, immediately after thawing (0 hour), and after 6 hours (**FIG. 17A**). Replating efficiency was evaluated at 0 and 6 hours post-thaw (**FIG. 17B**). The results showed that lower osmolality (~1900 mOsm/kg) achieved with reduced NaCl (0.36%) provided improved preservation outcomes compared to higher osmolality formulations.

[00276] These findings demonstrate the role of salt concentration and osmolality in maintaining cell viability and functionality during cryopreservation and subsequent thawing processes.

Example 7: Evaluation of Antioxidant Additives in Cell Cryopreservation and Delivery Solutions

[00277] This example describes experiments conducted to evaluate the effects of various antioxidant additives on cell preservation outcomes.

[00278] Neural cells (*i.e.*, dopaminergic precursor cells) were thawed and combined with test solutions containing different antioxidant combinations. The formulations compared included: 10% propylene glycol (PG) + 5% Dextran 40 alone, with 0.15% glutathione (GSH) + 0.1% isoleucine, or with 5% proline. The combined cell solutions were transferred to cryovials and cryopreserved using controlled rate freezing. The frozen cell vials were then transferred to LN2 vapour-phase storage.

[00279] Post-thaw cell viability was assessed at 0 and 6 hours after thawing using a NucleoCounter NC-200 (**FIG. 19A**). Replating efficiency was evaluated by plating the thawed cells onto culture dishes and analyzing cell adherence after 18-24 hours using NucLight Rapid Red staining (**FIG. 19B**). The data demonstrated that a combination of GSH and isoleucine showed potential benefits for replating efficiency.

[00280] Additional studies focused on evaluating the effects of GSH/isoleucine additives in formulations containing 12.5% PG + 1% Dextran 40 (**FIGS. 20A-20B**). Post-thaw viability and replating efficiency were assessed as described above. These results further validated the potential benefits of GSH/isoleucine incorporation in the cryopreservation and delivery solutions.

Example 8: Effect of pH on Cell Cryopreservation and Delivery Solutions

[00281] This example describes experiments conducted to evaluate the impact of pH on cell preservation outcomes.

[00282] Neural cells (i.e., dopaminergic neuronal precursor cells) were thawed and combined with test solutions at different pH values (pH 7.4 versus pH 7.8). The combined cell solutions were transferred to cryovials and cryopreserved using controlled rate freezing. The frozen cell vials were then transferred to LN2 vapour-phase storage.

[00283] Cell viability was assessed pre-cryopreservation, immediately after thawing (0 hour), and after 6 hours using a NucleoCounter NC-200 (**FIG. 21A**). Replating efficiency was evaluated by plating the thawed cells onto culture dishes and analyzing cell adherence after 18-24 hours using NucLight Rapid Red staining (**FIG. 21B**).

[00284] The data demonstrated that formulations at pH 7.8 provided enhanced replating efficiency compared to formulations at pH 7.4, while maintaining comparable cell viability. These results indicate that slightly alkaline conditions may be beneficial for maintaining cell functionality during cryopreservation and subsequent thawing processes.

Example 9: Development and Evaluation of One-Step versus Two-Step Cell Cryopreservation Methods

[00285] This example describes experiments conducted to compare different methods for preparing cell cryopreservation and delivery solutions.

[00286] Two distinct preparation methods were evaluated: a one-step direct addition method and an optimized two-step gradual addition method (**FIGS. 22A-22B**). In the one-step method, cells were directly resuspended in the final cryopreservation formulation before aliquoting and controlled rate freezing. In the two-step method, cells were first resuspended in an isotonic solution (Solution A) followed by gradual addition of a double-concentrated cryoprotectant solution (Solution B, 2X CPAs). The two-step approach was designed to minimize osmotic shock through gradual osmolality increase and avoid dilution effects from cell pellet volume.

[00287] The impact of these preparation methods was evaluated using an exemplary cell cryopreservation and delivery solution Formulation 47 (sometimes referred to as “F47” or #47). Formulation 47 includes 10% PG and 1% Dextran 40. Neuronal cells were prepared using either the one-step or two-step method, then cryopreserved using controlled rate freezing and stored in LN2 vapour-phase.

[00288] Post-thaw cell viability was assessed at 0 and 6 hours after thawing using a NucleoCounter NC-200 and replating efficiency was evaluated by plating the thawed cells onto culture dishes and analyzing cell adherence after 18-24 hours using NucLight Rapid Red staining (**FIGS. 23A-23B and 24A-24B**).

[00289] The results demonstrated that while both methods maintained comparable cell viability, the two-step preparation method provided improved replating efficiency. Without being bound to one theory, this enhancement may be attributed to reduced osmotic stress during the cell preparation process. Additionally, the two-step method ensured more precise final concentrations of cryoprotective agents by eliminating the dilution effect from cell pellet volume that occurs in the one-step approach.

[00290] These findings establish the two-step preparation method as an optimized approach for preparing cell cryopreservation and delivery solutions, particularly for sensitive cell types.

Example 10: Comparative Analysis of In-House and Commercial Cell Cryopreservation Solutions

[00291] This example describes experiments conducted to compare cryopreservation and delivery solutions, developed as described herein, with commercially available formulations.

[00292] Neural cells (*i.e.*, dopaminergic neuronal precursor cells) were prepared in different cryopreservation solutions including standard cell suspension for injection (CSI) (post-thaw dilution followed by washing and removing of cryopreservation medium), STEM-CELLBANKER, and various in-house formulations containing different combinations of propylene glycol (PG) and Dextran 40. The combined cell solutions were transferred to cryovials and cryopreserved using controlled rate freezing followed by storage in LN2 vapour-phase.

[00293] Initial comparative studies evaluated post-thaw cell viability (**FIGS. 25A-25B**) and replating efficiency (**FIGS. 26A-26B**) across multiple formulations. The data demonstrated that DMSO-free in-house formulations achieved comparable performance to commercial preparations containing DMSO.

[00294] Further studies specifically compared conventional approaches, STEM-CELLBANKER EX (a commercial DMSO-containing formulation), and the selected DMSO-free in-house formulation. Cell viability and replating efficiency measurements (**FIGS. 27A-27B**) showed similar preservation outcomes across all three approaches, validating the effectiveness of the DMSO-free formulation.

[00295] Additional detailed analysis compared standard STEM-CELLBANKER, STEM-CELLBANKER EX, and Formulation 50. Post-thaw viability (**FIG. 28A**) and live cell concentration measurements (**FIG. 28B**) demonstrated that the in-house Formulation 50 maintained comparable cell preservation outcomes to the commercial preparations while offering the advantages of a DMSO-free, thaw-and-inject format. These results demonstrate that the developed in-house formulations provide effective cell cryopreservation while eliminating DMSO and the need for post-thaw washing steps, representing potential advantages over currently available commercial solutions.

Example 11: Functional Assessment of Neural Cells Following Cryopreservation

[00296] This example describes experiments conducted to evaluate the functional characteristics and phenotype maintenance of neural cells following cryopreservation and delivery in the developed formulations.

[00297] Neural cells (*i.e.*, dopaminergic neuronal precursor cells) were cryopreserved in Standard STEM-CELLBANKER (Standard SCB), STEM-CELLBANKER EX (T&I SCB EX), XT-THRIVE, or Formulation 50. Following cryopreservation and thawing, cells were assessed for both on-target and off-target gene expression markers immediately after thaw (DIV0) and after 5 days in culture (DIV5).

[00298] Expression of on-target dopaminergic neuron markers (FOXA2, OTX2, LMX1A, and EN1) and off-target markers (CRABP1, PAX6, CD36, and KI67) was assessed by qPCR at DIV0 (**FIGS. 29A-29B**). After 5 days in culture, cells were re-analyzed for the same markers plus TH (tyrosine hydroxylase) as an additional on-target marker (**FIGS. 30A-30B**). Expression levels were normalized to geometric mean and presented as ΔCq values. The results demonstrated maintenance of appropriate gene expression profiles across all formulations tested.

[00299] Further functional assessment included evaluation of replating efficiency under different culture conditions. Dopaminergic neurons generated under 2D differentiation conditions were tested for replating efficiency with and without ROCK inhibitor (Rocki) following cryopreservation in different formulations (**FIGS. 31A-31B**). Similar studies were conducted with cells generated under 3D differentiation conditions (**FIGS. 32A-32B**). The data showed successful preservation of cell functionality and replating capacity across different culture formats.

[00300] These results demonstrate that the developed cryopreservation and delivery solutions maintain both phenotypic characteristics and functional properties of neural cells through the preservation process.

Example 12: *In vivo* assessment of cells administered by direct thaw-to-inject methods

[00301] *In vivo* experiments were conducted to evaluate cell engraftment using two different exemplary cell cryopreservation and delivery solutions: STEM CELLBANKER EX or Formulation No. 50.

[00302] Dopaminergic neuronal precursor cells were preserved in either STEM CELLBANKER EX or Formulation No. 50, frozen, thawed, and administered to mouse models. Post-sacrifice analysis assessed cell engraftment levels by fluorescent microscopy.

Table 3: Exemplary *In vivo* data of engrafted cells following direct thaw-to-inject administration

Graft Size	STEM CELLBANKER EX	Formulation No. 50
No graft	1	0
Small graft	2	3
Large graft	3	3

[00303] Results showed successful engraftment for both solutions. For STEM CELLBANKER EX, observations included one case with no graft, two cases with small grafts (10-200 cells per field of view (FOV)), and three cases with larger grafts (>200 cells per FOV). Formulation No. 50 demonstrated no cases without grafts, three cases with small grafts, and three cases with larger grafts.

[00304] The data indicates both solutions effectively preserved cell viability and supported successful engraftment following direct administration post-thaw.

EQUIVALENTS AND SCOPE, INCORPORATION BY REFERENCE

[00305] Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments described herein. It is understood that modifications which do not substantially affect the activity of the various embodiments of this disclosure are also provided within the description of the disclosure provided herein. The scope of the present disclosure is not intended to be limited to the above description, but rather is as set forth in the appended claims.

[00306] In the claims articles such as “a,” “an,” and “the” may mean one or more than one unless indicated to the contrary or otherwise evident from the context. Claims or descriptions that include “or” between one or more members of a group are considered satisfied if one, more than one, or all of the group members are present in, employed in, or otherwise relevant to a given product or process unless indicated to the contrary or otherwise

evident from the context. The disclosure includes embodiments in which exactly one member of the group is present in, employed in, or otherwise relevant to a given product or process. The disclosure also includes embodiments in which more than one, or all of the group members, are present in, employed in, or otherwise relevant to a given product or process.

[00307] Furthermore, it is to be understood that the disclosure encompasses all variations, combinations, and permutations in which one or more limitations, elements, clauses, descriptive terms, *etc.*, from one or more of the claims or from relevant portions of the description is introduced into another claim. For example, any claim that is dependent on another claim can be modified to include one or more limitations found in any other claim that is dependent on the same base claim. Furthermore, where the claims recite a composition, it is to be understood that methods of using the composition for any of the purposes disclosed herein are included, and methods of making the composition according to any of the methods of making disclosed herein or other methods known in the art are included, unless otherwise indicated or unless it would be evident to one of ordinary skill in the art that a contradiction or inconsistency would arise.

[00308] Where elements are presented as lists, *e.g.*, in Markush group format, it is to be understood that each subgroup of the elements is also disclosed, and any element(s) can be removed from the group. It should be understood that, in general, where the disclosure, or aspects of the embodiments, is/are referred to as that includes particular elements, features, steps, *etc.*, certain embodiments of the disclosure or aspects of the embodiments consist, or consist essentially of, such elements, features, steps, *etc.* Thus, for each embodiment of the disclosure that includes one or more elements, features, steps, *etc.*, the disclosure also provides embodiments that consist or consist essentially of those elements, features, steps, *etc.*

[00309] Where ranges are given, endpoints are included. Furthermore, it is to be understood that unless otherwise indicated or otherwise evident from the context and/or the understanding of one of ordinary skill in the art, values that are expressed as ranges can assume any specific value within the stated ranges in different embodiments of the disclosure, to the tenth of the unit of the lower limit of the range, unless the context clearly dictates otherwise. It is also to be understood that unless otherwise indicated or otherwise evident from the context and/or the understanding of one of ordinary skill in the art, values expressed as ranges can assume any subrange within the given range, wherein the endpoints of the

subrange are expressed to the same degree of accuracy as the tenth of the unit of the lower limit of the range.

[00310] In addition, it is to be understood that any particular embodiment of the present disclosure may be explicitly excluded from any one or more of the claims. Where ranges are given, any value within the range may explicitly be excluded from any one or more of the claims. Any embodiment, element, feature, application, or aspect of the compositions and/or methods of the disclosure, can be excluded from any one or more claims. For purposes of brevity, all of the embodiments in which one or more elements, features, purposes, or aspects is excluded are not set forth explicitly herein.

[00311] Throughout this disclosure various publications, patents, and sequence database entries are mentioned. The disclosures of these publications, patents, and sequence database entries, including those items listed above, are hereby incorporated by reference in their entirety as if each individual publication or patent was specifically and individually indicated to be incorporated by reference. In case of conflict, the present application, including any definitions herein, will control.

[00312] Although the disclosure has been described with reference to the examples provided above, it should be understood that various modifications can be made without departing from the scope of the disclosure. Accordingly, the above examples are intended to illustrate but not limit the present disclosure.

CLAIMS

What is claimed is:

1. A cell cryopreservation and delivery solution, the solution comprising:
about 25 mM to about 280 mM of one or more energy source components;
about 10 mM to about 200 mM of one or more salts; and
about 1% wt to about 30% wt of one or more cryoprotectants.
2. The cell cryopreservation and delivery solution of claim 1, further comprising about 5 mM to about 40 mM of one or more pH buffers.
3. The cell cryopreservation and delivery solution of claim 2, wherein the one or more pH buffers comprise 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 3-(N-morpholino)propanesulfonic acid (MOPS), 2-(N-morpholino) ethanesulfonic acid (MES), bicarbonate, or phosphates.
4. The cell cryopreservation and delivery solution of any one of claims 1 to 3, further comprising about 0.01% wt to about 25% wt of one or more stabilizing agents.
5. The cell cryopreservation and delivery solution of claim 4, wherein the one or more stabilizing agents comprise dextran, poloxamer, polyvinylpyrrolidone, one or more antioxidants, isoleucine, or albumin.
6. The cell cryopreservation and delivery solution of claim 5, wherein the one or more antioxidants comprise glutathione or proline.
7. The cell cryopreservation and delivery solution of claim 6, wherein the one or more antioxidants comprise glutathione.

8. The cell cryopreservation and delivery solution of any of claims 1 to 7, wherein the one or more cryoprotectants comprise propylene glycol, glycerol, or dimethyl sulfoxide (DMSO).
9. The cell cryopreservation and delivery solution of claim 8, wherein the one or more cryoprotectants comprise propylene glycol.
10. The cell cryopreservation and delivery solution of any one of claims 1 to 9, wherein the one or more energy source components comprise dextrose, glucose, sucrose, mannitol or trehalose.
11. The cell cryopreservation and delivery solution of any one of claims 1 to 10, wherein the one or more salts comprise one or more of calcium chloride, magnesium chloride, potassium chloride, sodium chloride, or sodium phosphate.
12. The cell cryopreservation and delivery solution of claim 11, wherein the solution comprises:
- about 120 mM to about 190 mM glucose;
 - about 4 mM to about 40 mM HEPES;
 - about 0.1 mM to about 2.5 mM calcium chloride;
 - about 0.1 mM to about 1.5 mM magnesium chloride;
 - about 4.0 mM to about 6.5 mM potassium chloride;
 - about 20 mM to about 150 mM sodium chloride;
 - about 0.1% wt to about 20% wt Dextran; and
 - about 5% wt to about 25% wt of the one or more cryoprotectants.
13. The cell cryopreservation and delivery solution of claim 12, wherein the solution comprises:
- about 145 mM to about 165 mM glucose;
 - about 10 mM to about 15 mM HEPES;
 - about 0.1 mM to about 2.5 mM calcium chloride;
 - about 0.5 mM to about 1.0 mM magnesium chloride;
 - about 4.0 mM to about 6.0 mM potassium chloride;
 - about 60 mM to about 80 mM sodium chloride;

about 0.1% wt to about 10% wt Dextran; and
about 10% wt to about 15% wt of one or more cryoprotectants.

14. The cell cryopreservation and delivery solution of any one of claims 1 to 13, wherein the one or more cryoprotectants comprise dimethyl sulfoxide (DMSO).
15. The cell cryopreservation and delivery solution of any of claims 1 to 14, wherein the one or more cryoprotectants comprise propylene glycol.
16. The cell cryopreservation and delivery solution of any one of claims 1 to 15, wherein the solution does not include any components of animal origin.
17. The cell cryopreservation and delivery solution of any one of claims 1 to 16, wherein the solution does not include one or more of zinc sulfate, and ferric nitrate.
18. The cell cryopreservation and delivery solution of any one of claims 1 to 17, wherein the solution comprises a pH between about 7.0 and about 8.0.
19. The cell cryopreservation and delivery solution of claim 18, wherein the pH is less than 7.8.
20. The cell cryopreservation and delivery solution of any one of claims 1 to 19, wherein the solution does not include sodium pyruvate.
21. The cell cryopreservation and delivery solution of any one of claims 1 to 20, wherein the cell cryopreservation and cell delivery solution enables cryopreservation of a plurality of cells at a therapeutically effective dose.
22. The cell cryopreservation and delivery solution of any one of claims 1 to 21, wherein the cell cryopreservation and delivery solution enables stable storage of a plurality of cells for up to at least about 6 hours at 4 °C.
23. A cell suspension for cryopreservation and administration to a subject, the suspension comprising:

a plurality of cells;
about 25 mM to about 280 mM of one or more energy source components;
about 10 mM to about 200 mM of one or more salts; and
about 1% wt to about 30% wt of one or more cryoprotectants.

24. The cell suspension of claim 23, wherein the plurality of cells comprise human pluripotent stem cells, embryonic stem cells, cardiac cells, microglial cells, T-cells, or neural cells.
25. The cell suspension of claim 24, wherein the plurality of cells comprise neural cells and wherein the neural cells comprise dopaminergic neuronal precursor cells.
26. The cell suspension of any one of claims 23 to 25, wherein the plurality of cells comprises a population of about 10^4 cells/mL to about 10^{10} cells/mL.
27. The cell suspension of any one of claim 26, wherein the plurality of cells comprises a range of about 10^5 cells/mL to about 1.5×10^8 cells/mL.
28. The cell suspension of any one of claims 23 to 27, wherein the suspension does not require contacting the plurality of cells with a cell wash solution prior to administration to the subject.
29. The cell suspension of claim 28, wherein the cell wash solution comprises a phosphate buffer saline (PBS), Bio-Plex Pro™ Cell Signaling Wash Buffer, or Cultrex™ 3-D Cell Wash Buffer.
30. The cell suspension of any one of claims 23 to 29, further comprising about 5 mM to about 40 mM of one or more pH buffers.
31. The cell suspension of any one of claims 23 to 30, further comprising about 0.01% wt to about 25% wt of one or more stabilizing agents.

32. The cell suspension of any one of claims 23 to 31, wherein the one or more cryoprotectants comprise propylene glycol, glycerol, or dimethyl sulfoxide (DMSO).
33. The cell suspension of any one of claims 23 to 32, wherein the one or more cryoprotectants comprise propylene glycol.
34. The cell suspension of any one of claims 23 to 33, wherein the one or more energy source components comprise dextrose, glucose, sucrose, mannitol or trehalose.
35. The cell suspension of any one of claims 30 to 34, wherein the one or more pH buffers comprise 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 3-(N-morpholino)propanesulfonic acid (MOPS), 2-(N-morpholino) ethanesulfonic acid (MES), bicarbonate, or phosphates.
36. The cell suspension of any one of claims 23 to 35, wherein the one or more salts comprise one or more of calcium chloride, magnesium chloride, potassium chloride, sodium chloride, or sodium phosphate.
37. The cell suspension of any one of claims 31 to 36, wherein the one or more stabilizing agents comprise dextran, poloxamer, polyvinylpyrrolidone, one or more antioxidants, or albumin.
38. The cell suspension of any one of claims 27 to 37, wherein the suspension comprises:
about 120 mM to about 190 mM glucose;
about 4 mM to about 40 mM HEPES;
about 0.1 mM to about 2.5 mM calcium chloride;
about 0.1 mM to about 1.5 mM magnesium chloride;
about 4.0 mM to about 6.5 mM potassium chloride;
about 20 mM to about 150 mM sodium chloride;
about 0.1% wt to about 20% wt Dextran; and
about 5% wt to about 25% wt of the one or more cryoprotectants.
39. The cell suspension of claim 38, wherein the suspension comprises:
about 145 mM to about 165 mM glucose;

about 10 mM to about 15 mM HEPES;
about 0.1 mM to about 2.5 mM calcium chloride;
about 0.5 mM to about 1.0 mM magnesium chloride;
about 4.0 mM to about 6.0 mM potassium chloride;
about 60 mM to about 80 mM sodium chloride;
about 0.1% wt to about 10% wt Dextran; and
about 10% wt to about 15% wt of one or more cryoprotectants.

40. The cell suspension of any of claims 23 to 39, wherein the one or more cryoprotectants comprise dimethyl sulfoxide (DMSO).
41. The cell suspension of any of claims 23 to 40, wherein the one or more cryoprotectants comprise propylene glycol.
42. The cell suspension of any one of claims 23 to 41, wherein the suspension does not include any components of animal origin.
43. The cell suspension of any one of claims 23 to 42, wherein the suspension does not include one or more of zinc sulfate, and ferric nitrate.
44. The cell suspension of any one of claims 23 to 43, wherein the cell suspension comprises a pH between about 7.0 and about 8.0.
45. The cell suspension of claim 44, wherein the pH is below 7.8.
46. The cell suspension of any one of claims 37 to 45, wherein the cell suspension does not include sodium pyruvate.
47. The cell suspension of any one of claims 37 to 46, wherein the one or more antioxidants comprise glutathione or proline.
48. The cell suspension of claim 47, wherein the one or more antioxidants comprise glutathione.

49. The cell suspension of any one of claims 23 to 48, wherein the cell suspension enables cryopreservation of the plurality of cells at a therapeutically effective dose.
50. The cell suspension of any one of claims 23 to 49, wherein the cell suspension enables stable storage of the plurality of cells for up to at least about 6 hours at 4 °C.
51. A method of preparing a cell suspension for cryopreservation and administration to a subject, the method comprising:
- adding the cell cryopreservation and delivery solution of any one of claims 1 to 22 to a plurality of cells in a container; and
 - mixing the plurality of cells and the cell cryopreservation and delivery solution to form a cell suspension.
52. The method of claim 51, wherein the plurality of cells comprises a population of about 10^4 cells/mL to about 10^{10} cells/mL.
53. The method of claim 51 or claim 52, wherein the plurality of cells comprises a range of about 10^5 cells/mL to about 1.5^8 cells/mL.
54. The method of any one of claims 51 to 53, wherein the cell suspension has a volume of about 0.1 mL to about 20 mL.
55. The method of any one of claims 51 to 54, wherein the plurality of cells has a variability in dose sampling of less than about 25% in the container.
56. The method of any one of claims 51 to 55, wherein the plurality of cells are differentiated midbrain dopaminergic neuronal cells, or a precursor thereof.
57. The method of any one of claims 51 to 56, further comprising cryopreserving the cell suspension.
58. The method of claim 57, further comprising thawing the cryopreserved cell suspension, and administering the thawed cell suspension to a subject.

59. The method of claim 58, wherein the method does not comprise washing the cells with a cell wash solution after thawing the cells.
60. The method of claim 59, wherein the cell wash solution comprises phosphate buffer saline (PBS), Bio-Plex Pro™ Cell Signaling Wash Buffer, or Cultrex™ 3-D Cell Wash Buffer.
61. A method of treating a subject, the method comprising:
thawing the cell suspension of any one of claims 23 to 50; and
administering a dose of the cell suspension to the subject,
wherein the method does not comprise a wash step.
62. The method of claim 61, wherein the cells are differentiated midbrain dopaminergic neuronal cells.
63. The method of claim 61 or claim 62, wherein the plurality of cells comprises a population of about 10^4 cells/mL to 10^{10} cells/mL.
64. The method of claim 63, wherein the population comprises about 10^5 cells/mL to about 1.5×10^8 cells/mL.
65. The method of any one of claims 61 to 64, further comprising the step of administering one or more additional doses of the cell suspension.
66. The method of any one of claims 61 to 65, wherein the wash step comprises washing with a cell wash buffer comprising a phosphate buffer saline (PBS), Bio-Plex Pro™ Cell Signaling Wash Buffer, or Cultrex™ 3-D Cell Wash Buffer.
67. The method of any one of claims 61 to 66, wherein the administering comprises loading a dose delivery device with the dose of the cell suspension, and injecting the dose to the subject.
68. The method of claim 67, wherein the injecting occurs directly into a portion of the subject's brain.

69. The method of claim 68, wherein the portion comprises a putamen.
70. The method of claim 67, wherein the dose delivery device is a syringe.
71. The method of any one of claims 61 to 70, wherein the subject is a human.
72. The method of claim 71, wherein the subject has Parkinson's disease.
73. The method of claim 72, wherein the Parkinson's disease is advanced Parkinson's disease.
74. A method of treating a subject, the method comprising:
thawing a cell suspension comprising about 2% wt to about 30% wt of one or more cryoprotectants; and
administering a dose of the cell suspension to the subject.
75. The method of claim 74, wherein the method does not comprise a cell wash step.
76. The method of claim 74 or 75, wherein the method does not comprise diluting the cell suspension prior to the administering.
77. The method of any of claim 74 to 76, wherein the cell suspension comprises:
about 25 mM to about 280 mM of one or more energy source components;
about 10 mM to about 200 mM of one or more salts; and
about 1% wt to about 30% wt of the one or more cryoprotectants.
78. The method of claim 77, wherein the cell suspension further comprises about 5 mM to about 40 mM of one or more pH buffers.
79. The method of claim 77 to 78, wherein the cell suspension further comprises one or more pH buffers, wherein the one or more pH buffers comprise 4-(2-hydroxyethyl)-1-

piperazineethanesulfonic acid (HEPES), 3-(N-morpholino)propanesulfonic acid (MOPS), 2-(N-morpholino) ethanesulfonic acid (MES), bicarbonate, or phosphates.

80. The method of any of claims 77 to 79, further comprising about 0.01% wt to about 25% wt of one or more stabilizing agents.

81. The method of claim 80, wherein the one or more stabilizing agents comprise dextran, poloxamer, polyvinylpyrrolidone, one or more antioxidants, or recombinant human serum albumin.

82. The method of any of claims 74 to 81, wherein the one or more cryoprotectants comprise propylene glycol, glycerol, or dimethyl sulfoxide (DMSO).

83. The method of any of claims 74 to 81, wherein the one or more cryoprotectants comprise propylene glycol.

84. The method of any of claims 77 to 83, wherein the one or more energy source components comprise dextrose, glucose, sucrose, mannitol or trehalose.

85. The method of any of claims 77 to 83, wherein the one or more salts comprise one or more of calcium chloride, magnesium chloride, potassium chloride, sodium chloride, or sodium phosphate.

86. The method of claim 85, wherein the cell suspension comprises:
about 120 mM to about 190 mM glucose;
about 4 mM to about 40 mM HEPES;
about 0.1 mM to about 2.5 mM calcium chloride;
about 0.1 mM to about 1.5 mM magnesium chloride;
about 4.0 mM to about 6.5 mM potassium chloride;
about 20 mM to about 150 mM sodium chloride;
about 0.1% wt to about 20% wt Dextran; and
about 5% wt to about 25% wt of the one or more cryoprotectants.

87. The method of any of claims 87 to 86, wherein the cell suspension comprises:

about 145 mM to about 165 mM glucose;
about 10 mM to about 15 mM HEPES;
about 0.1 mM to about 2.5 mM calcium chloride;
about 0.5 mM to about 1.0 mM magnesium chloride;
about 4.0 mM to about 6.0 mM potassium chloride;
about 60 mM to about 80 mM sodium chloride;
about 0.1% wt to about 10% wt Dextran; and
about 10% wt to about 15% wt of one or more cryoprotectants.

88. The method of claims 74 to 76, wherein the cell suspension comprises a cryopreservation solution comprising one or more cryoprotectants selected from the group consisting of dimethyl sulfoxide (DMSO), DMSO-free cryoprotectants, and combinations thereof.

89. The method of claim 88, wherein the cryopreservation solution comprises:
about 5% to about 15% DMSO; or
about 5% to about 15% propylene glycol and about 1% to about 5% Dextran 40.

90. The method of claim 88 or 89, wherein the cryopreservation solution comprises one of STEM-CELLBANKER™ EX, STEM-CELLBANKER™, STEM-CELLBANKER™ DMSO Free, or XT-THRIVE™.

91. A method of preparing a cell cryopreservation and delivery solution, the method comprising:

preparing a first solution comprising about 0.2% wt to about 20% wt dextran and about 20% wt to about 30% wt of one or more cryoprotectants;

preparing a second solution comprising:

about 145 mM to about 165 mM glucose,
about 10 mM to about 15 mM HEPES,
about 0.1 mM to about 2.5 mM calcium chloride,
about 0.5 mM to about 1.0 mM magnesium chloride,
about 4.0 mM to about 6.0 mM potassium chloride, and
about 60 mM to about 80 mM sodium chloride; and

combining substantially equal volumes of the first solution and the second solution to form the cell cryopreservation and delivery solution.

92. A method of preparing a final cell suspension for cryopreservation and delivery to a subject, the method comprising:

suspending a plurality of cells in an isotonic solution to form a cell suspension;

preparing a concentrated cryoprotectant solution comprising about 20% wt to about 30% wt of one or more cryoprotectants; and

gradually adding the concentrated cryoprotectant solution to the cell suspension to form the final cell suspension.

93. The method of claim 92, wherein the isotonic solution comprises:

about 145 mM to about 165 mM glucose,

about 10 mM to about 15 mM HEPES,

about 0.1 mM to about 2.5 mM calcium chloride,

about 0.5 mM to about 1.0 mM magnesium chloride,

about 4.0 mM to about 6.0 mM potassium chloride, and

about 60 mM to about 80 mM sodium chloride.

94. The method of claim 92 or 93, wherein the concentrated cryoprotectant solution further comprises about 0.2% wt to about 20% wt dextran.